

FIG. 1A

```

1  agggagagggc agtgaccatg aaggctgtgc tgcttgccct gttgatggca
51  ggcttggccc tgcagccagg cactgccctg ctgtgctact cctgcaaagc
101 ccaggtgagc aacgaggact gcctgcaggt ggagaactgc acccagctgg
151 gggagcagtg ctggaccgcg cgcattccgc cagttggcct cctgaccgtc
201 atcagcaaag gctgcagctt gaactgcgtg gatgactcac aggactacta
251 cgtgggcaag aagaacatca cgtgctgtga caccgacttg tgcaacgcca
301 gcggggccca tgccctgcag ccggctgccc ccattccttg gctgctccct
351 gcactcggcc tgctgctctg gggacccggc cagctatagg ctctgggggg
401 ccccgctgca gcccacactg ggtgtggtgc cccaggcctt tgtgccactc
451 ctcacagaac ctggcccagt gggagcctgt cctggttcct gaggcacatc
501 ctaacgcaag tttgaccatg tatgtttgca ccccttttcc ccnaaccctg
551 accttcccat gggccttttc caggattccn accnggcaga tcagttttag
601 tganacanat ccgcntgcag atggcccctc caacentttt tgttgntggt
651 tccatggccc agcattttcc acccttaacc ctgtgttcag gcacttnttc
701 cccaggaag ccttccctgc ccacccatt tatgaattga gccaggtttg
751 gtccgtggtg tccccgcac ccagcagggg acaggcaatc aggagggccc
801 agtaaaggct gagatgaagt ggactgagta gaactggagg acaagagttg
851 acgtgagttc ctgggagttt ccagagatgg ggcctggagg cctggaggaa
901 ggggccaggc ctcacatttg tgggntccc gaatggcagc ctgagcacag
951 cgtaggccct taataaacac ctgttgata agccaaaaaa aaaaaaaa

```

FIG. 1B

```

MKAVLLALLMAGLALQPGTALLCYSCKAQVSNECLQV
ENCTQLGEQCWTARIRAVGLLTVISKGCSLNCVDDS
QDYVVGKKNITCCDIDLNASGAHALQPAAAILALLPAL
GLLLWGPQQL

```

FIG. 2

1 ATGAAGACAGTTTTTTTTATCCTGCTGGCCACCTACTTAGCCCTGCATCCAGGTGCTGCT
 -----+-----+-----+-----+-----+-----+ 60
 TACTTCTGTCAAAAAAAAAATAGGACGACCGGTGGATGAATCGGGACGTAGGTCCACGACGA

 M K T V F F I L L A T Y L A L H P G A A

 61 CTGCAGTGCTATTCATGCACAGCACAGATGAACAACAGAGACTGTCTGAATGTACAGAAC
 -----+-----+-----+-----+-----+-----+ 120
 GACGTCACGATAAGTACGTGTCGTGTCTACTTGTGTCTCTGACAGACTTACATGTCTTG

 L Q C Y S C T A Q M N N R D C L N V Q N

 121 TGCAGCCTGGACCAGCACAGTTGCTTTACATCGCGCATCCGGGCCATTGGACTCGTGACA
 -----+-----+-----+-----+-----+-----+ 180
 ACGTCGGACCTGGTCGTGTCAACGAAATGTAGCGCGTAGGCCCGGTAACCTGAGCACTGT

 C S L D Q H S C F T S R I R A I G L V T

 181 GTTATCAGTAAGGGCTGCAGCTCACAGTGTGAGGATGACTCGGAGAACTACTATTTGGGC
 -----+-----+-----+-----+-----+-----+ 240
 CAATAGTCATTCCCGACGTCGAGTGTCACTCCTACTGAGCCTCTTGATGATAAACCCG

 V I S K G C S S Q C E D D S E N Y Y L G

 241 AAGAAGAACATCACGTGCTGCTACTCTGACCTGTGCAATGTCAACGGGGCCCACACCCTG
 -----+-----+-----+-----+-----+-----+ 300
 TTCTTCTTGTAGTGACGACGATGAGACTGGACACGTTACAGTTGCCCCGGGTGTGGGAC

 K K N I T C C Y S D L C N V N G A H T L

 301 AAGCCACCCACCCCTGGGGCTGCTGACCGTGCTCTGCAGCCTGTTGCTGTGGGGCTCC
 -----+-----+-----+-----+-----+-----+ 360
 TTCGGTGGGTGGTGGGACCCGACGACTGGCAGGACGTCGGACAACGACACCCCGAGG

 K P P T T L G L L T V L C S L L L W G S

 361 AGCCGTCTGTAGGCTCTGGGAGAGCCTACCATAGCCCGATTGTGAAGGGATGAGCTGCAC
 -----+-----+-----+-----+-----+-----+ 420
 TCGGCAGACATCCGAGACCCTCTCGGATGGTATCGGGCTAACACTTCCCTACTCGACGTG

 S R L *

 421 TCCAÇCCACCCCCACACAGG
 -----+-----+-----+ 441
 AGGTGGGGTGGGGGTGTGTCC

1 M K I F L P V L L A A L L G V E R A S S hSCA-2
1 M K A V L L A L L M A G L A L Q P G T A hPSCA
1 M K T V L F L L L A T Y L A L H P G A A mPSCA

21 L M C F S C L N Q K S N* L Y C L K P T I
21 L L C Y S C K A Q V S N* E D C L Q V E N*
21 L Q C Y S C T A Q M N N* R D C L N V Q N*

41 C S D Q D N Y C V T V S A S A G I G N L
41 C T Q L G E Q C W T A R I R A V G L L T
41 C S L D Q H S C F T S R I R A I G L V T

61 V T F G H S L S K T C S P A C P I P E G
61 V - - - - I S K G C S L N C V D D S Q
61 V - - - - I S K G C S S Q C E D D S E

81 V N V G V A S M G I S C C Q S F L C N* F
76 D Y Y V G K K - N* I T C C D T D L C N* A
76 N Y Y L G K K - N* I T C C Y S D L C N* V

101 S A A D G G L R A S V T L L G A G L L L
95 S G A H A L Q P A A A I L A L L P A L G
95 N G A H T L K P P T T L G L L T V L C S

121 S L L P A L L R F G P
115 L L L W G P G Q L - -
115 L L L W G S S R L - -

FIG. 4

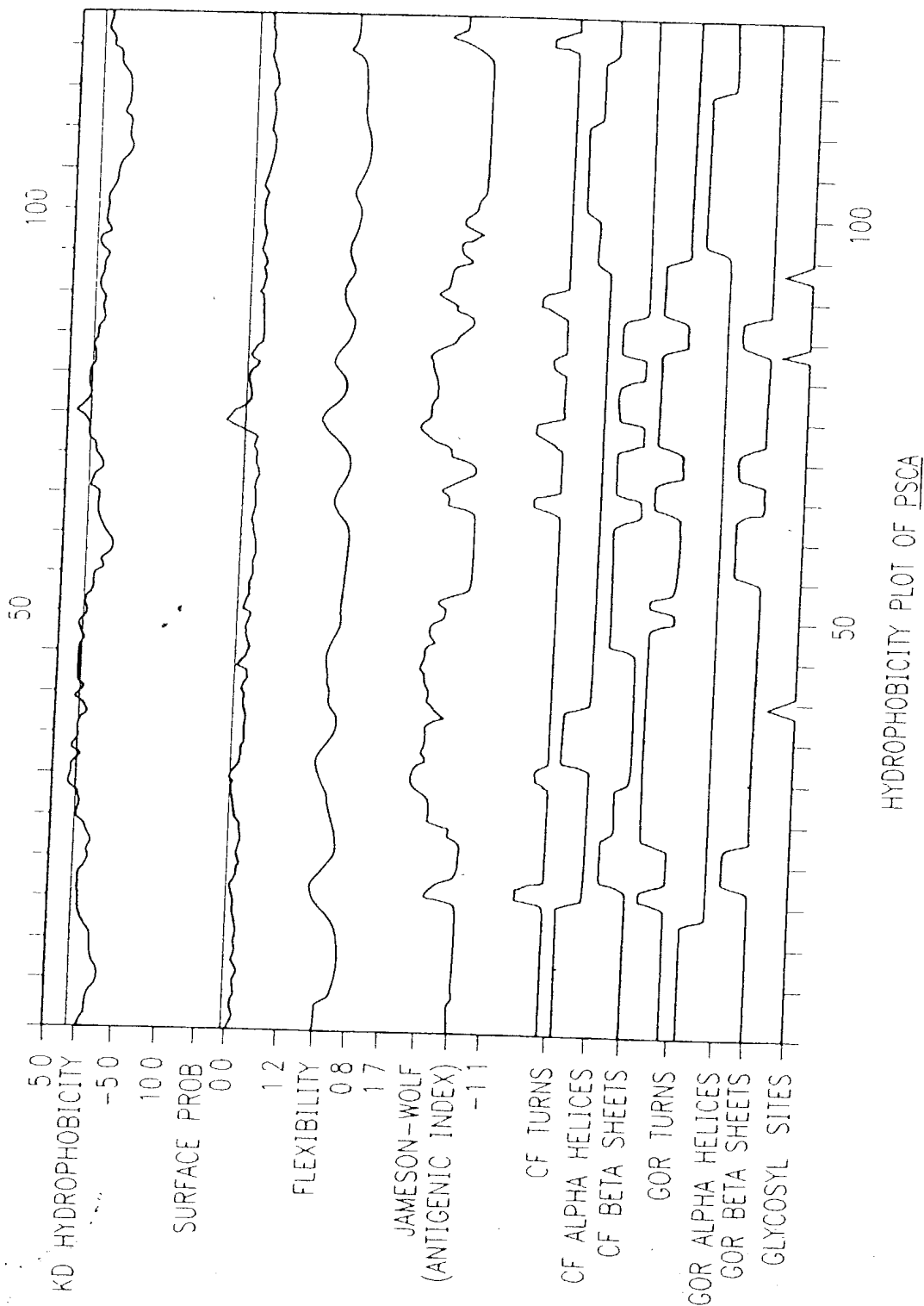


FIG. 5

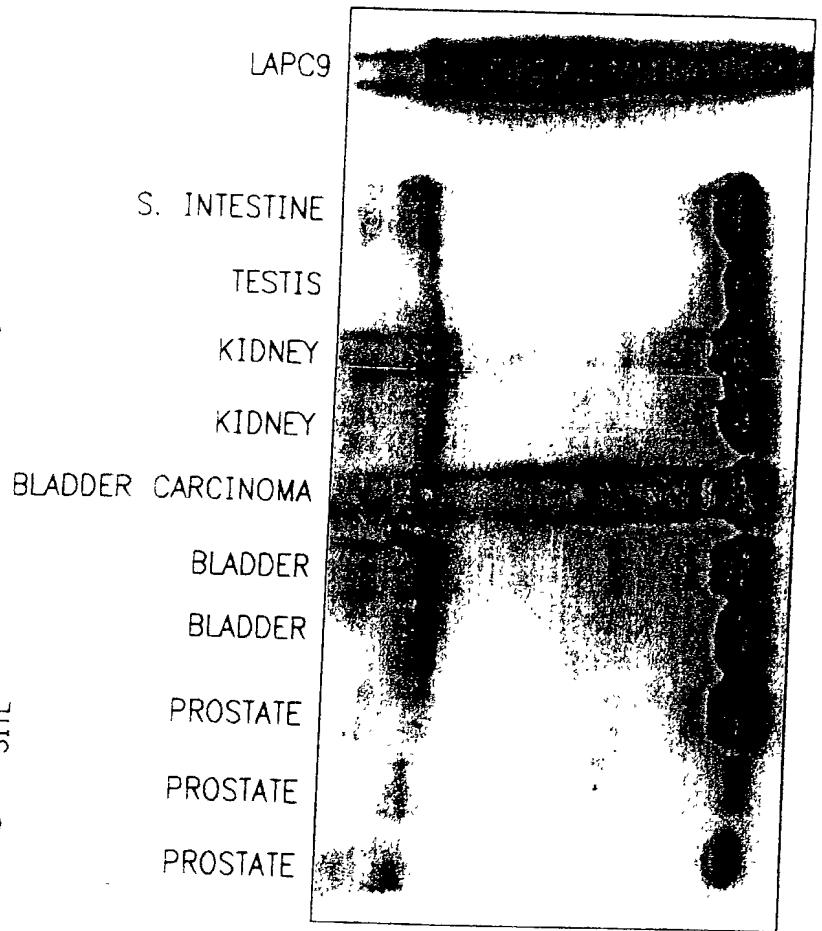
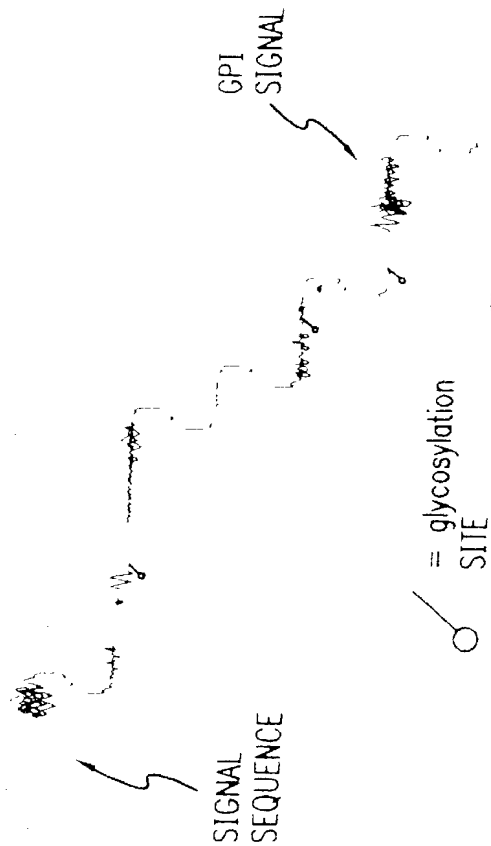


FIG. 6

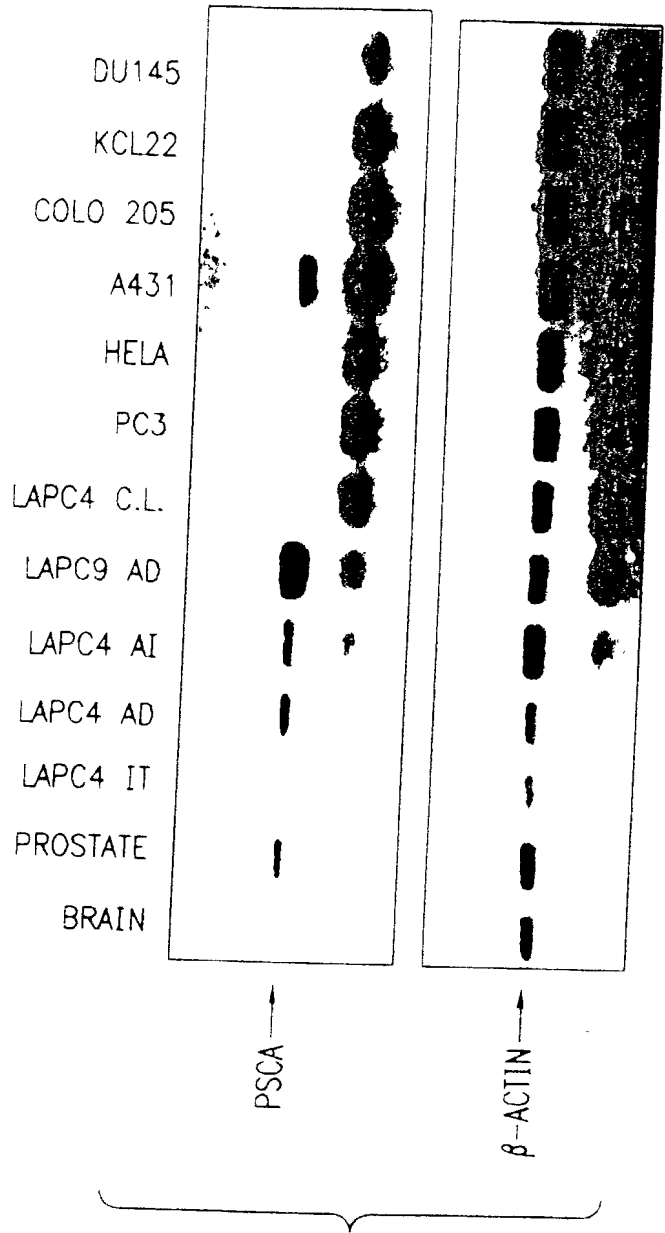
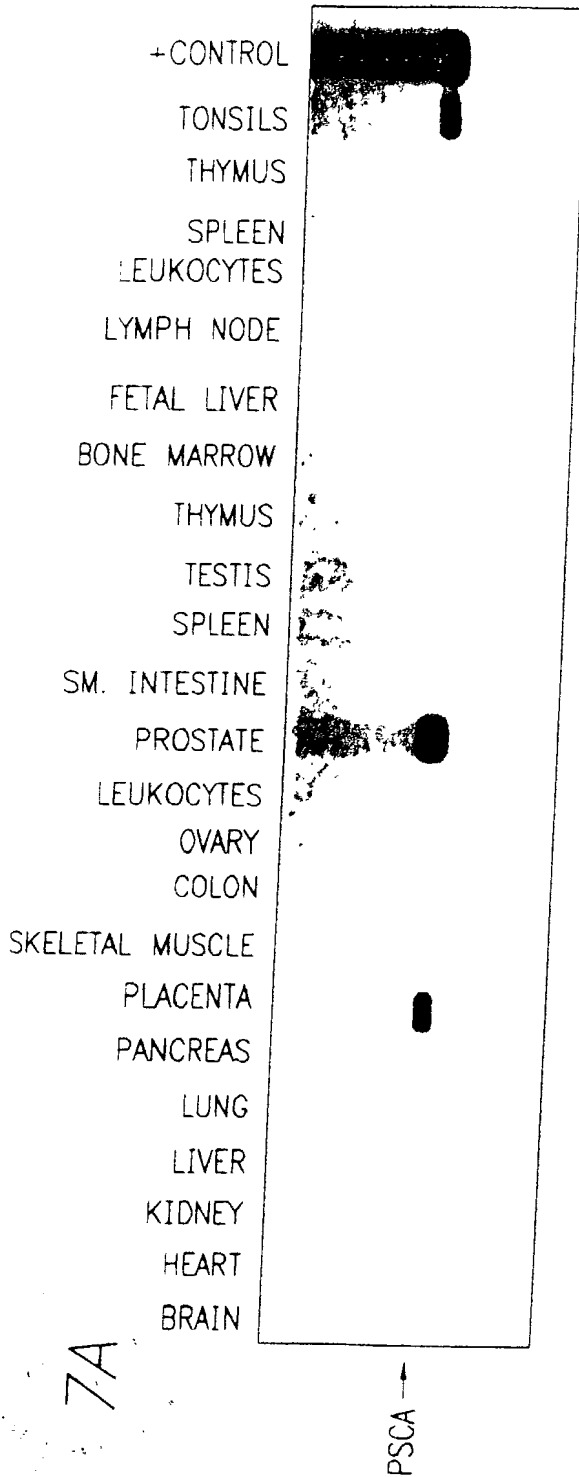


FIG. 8A

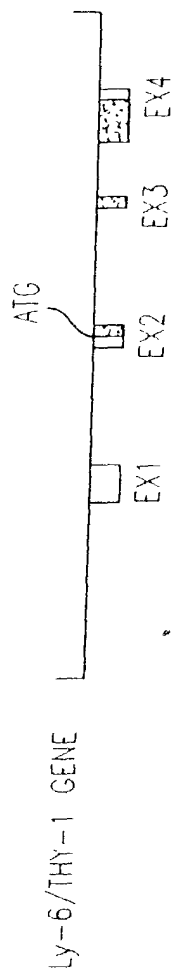


FIG. 8B

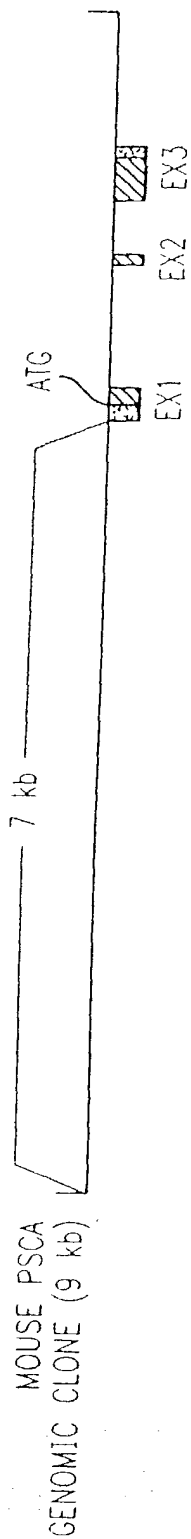
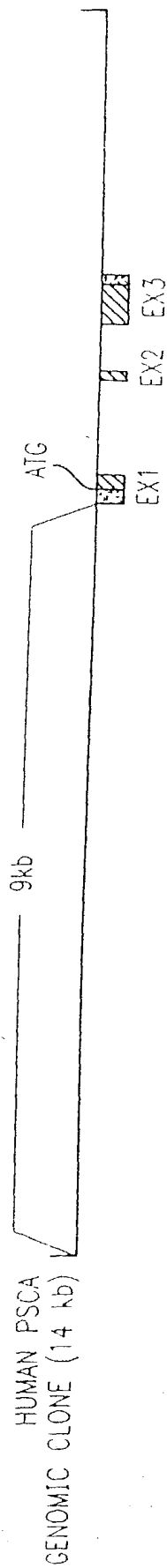


FIG. 8C



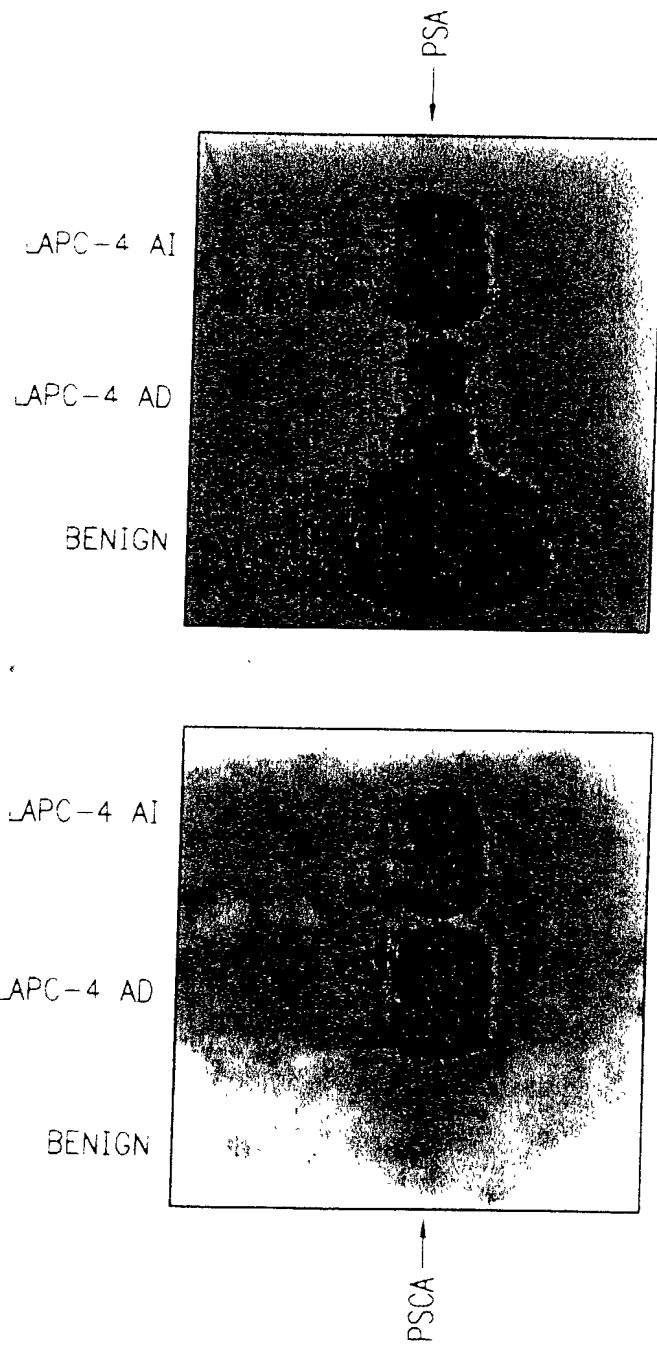


FIG. 9A

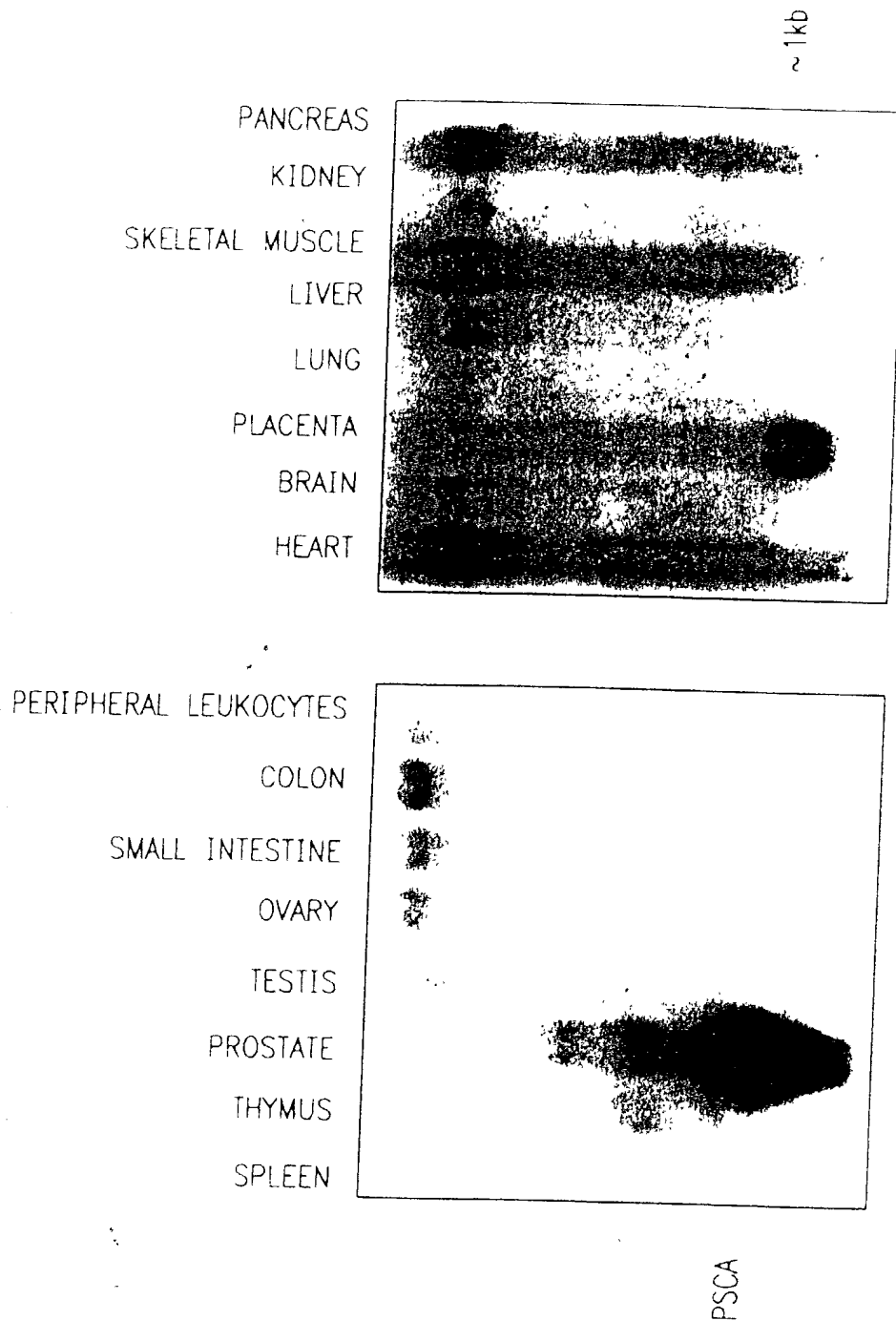
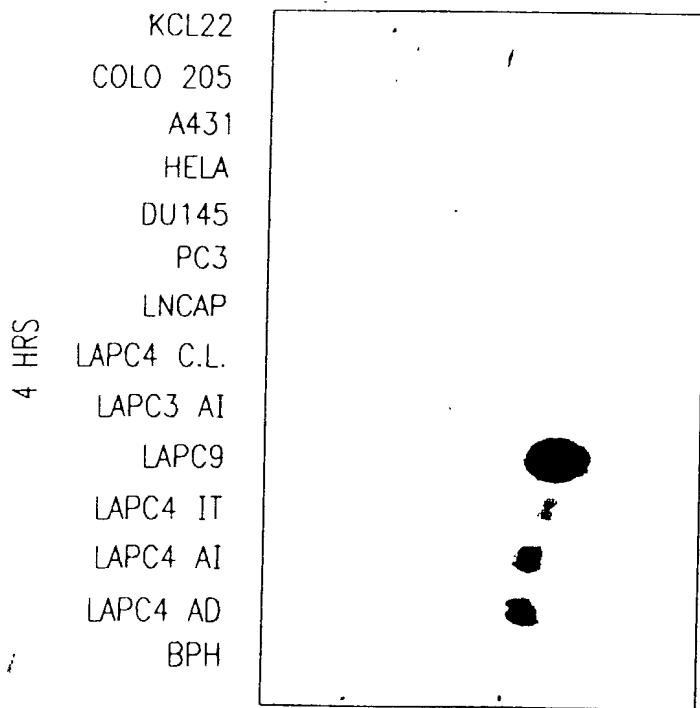
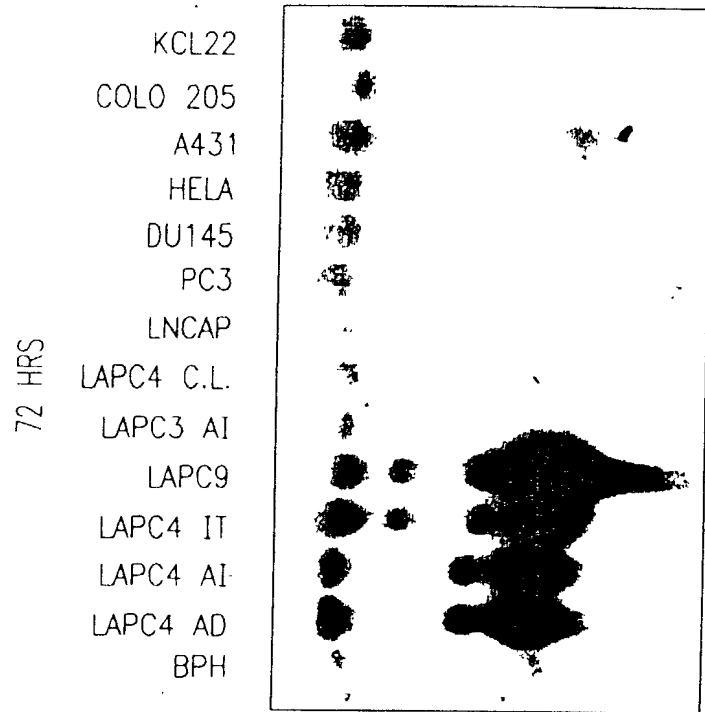


FIG. 9B

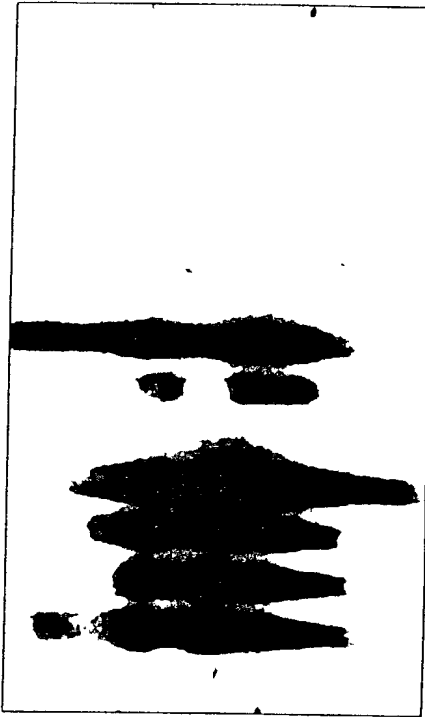


PSCA

FIG. 10A

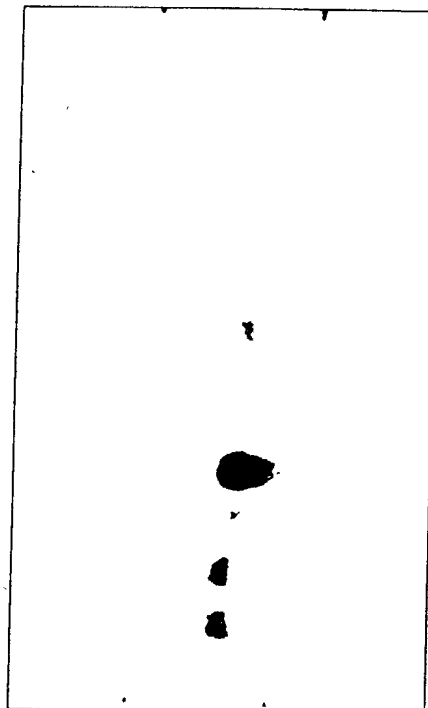
72 HRS

KCL22
 COLO 205
 A431
 HELA
 DU145
 PC3
 LNCAP
 LAPC4 C.L.
 LAPC3 AI
 LAPC9
 LAPC4 IT
 LAPC4 AI
 LAPC4 AD
 BPH



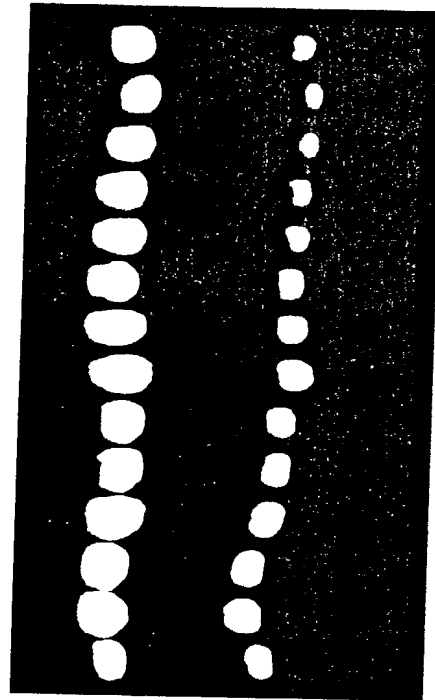
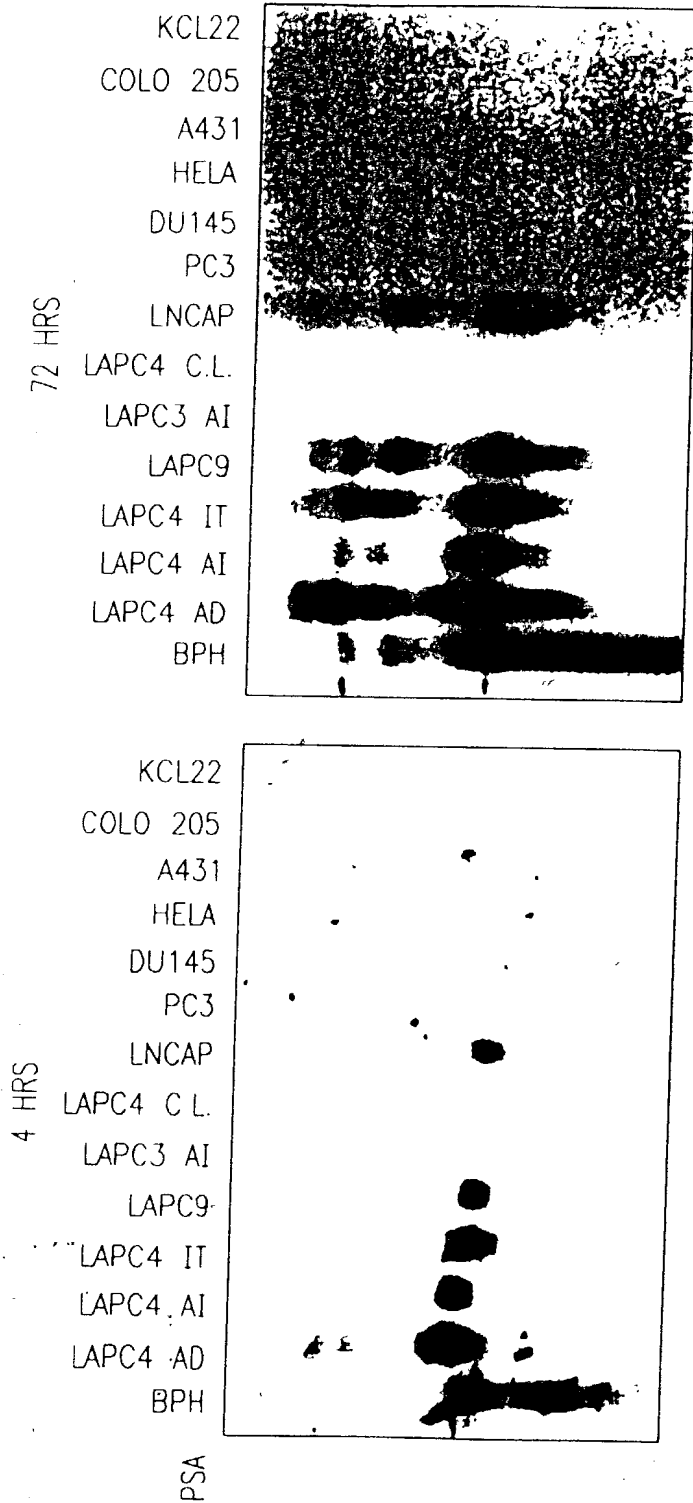
4 HRS

KCL22
 COLO 205
 A431
 HELA
 DU145
 PC3
 LNCAP
 LAPC4 C.L.
 LAPC3 AI
 LAPC9
 LAPC4 IT
 LAPC4 AI
 LAPC4 AD
 BPH



PSM

FIG. 10B



ETBR

FIG. 10C

FIG. 11A

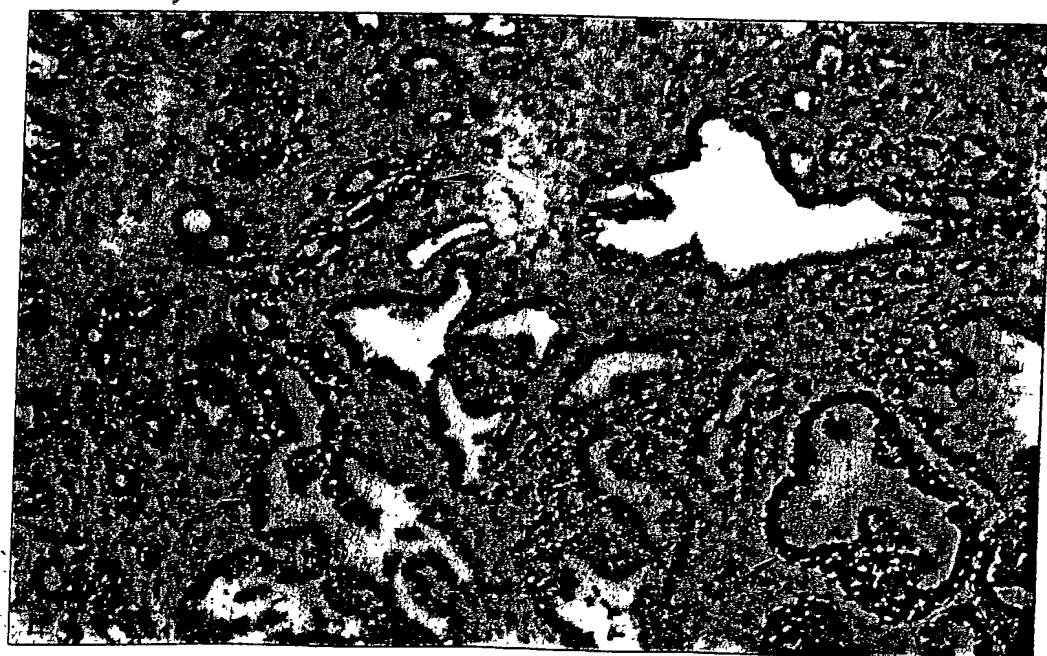


FIG. 11B

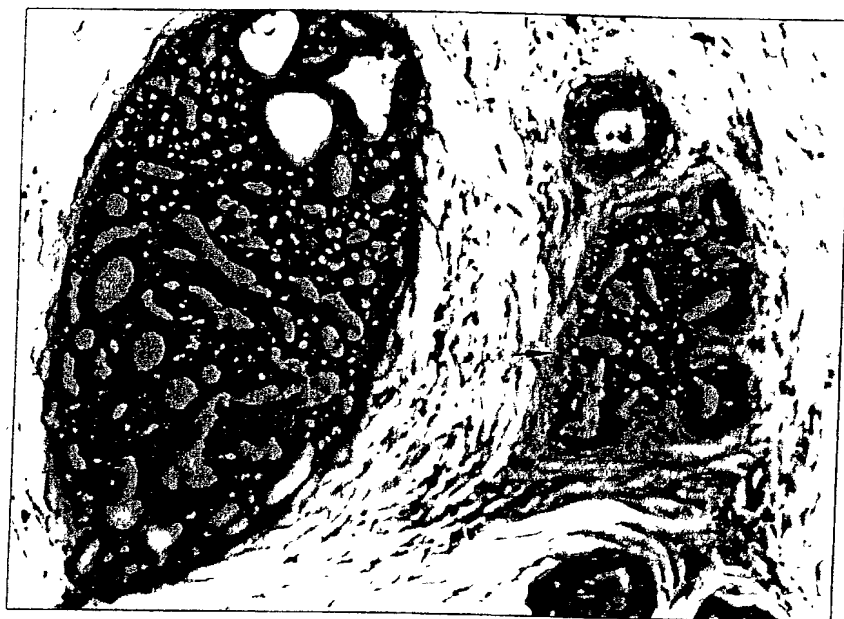
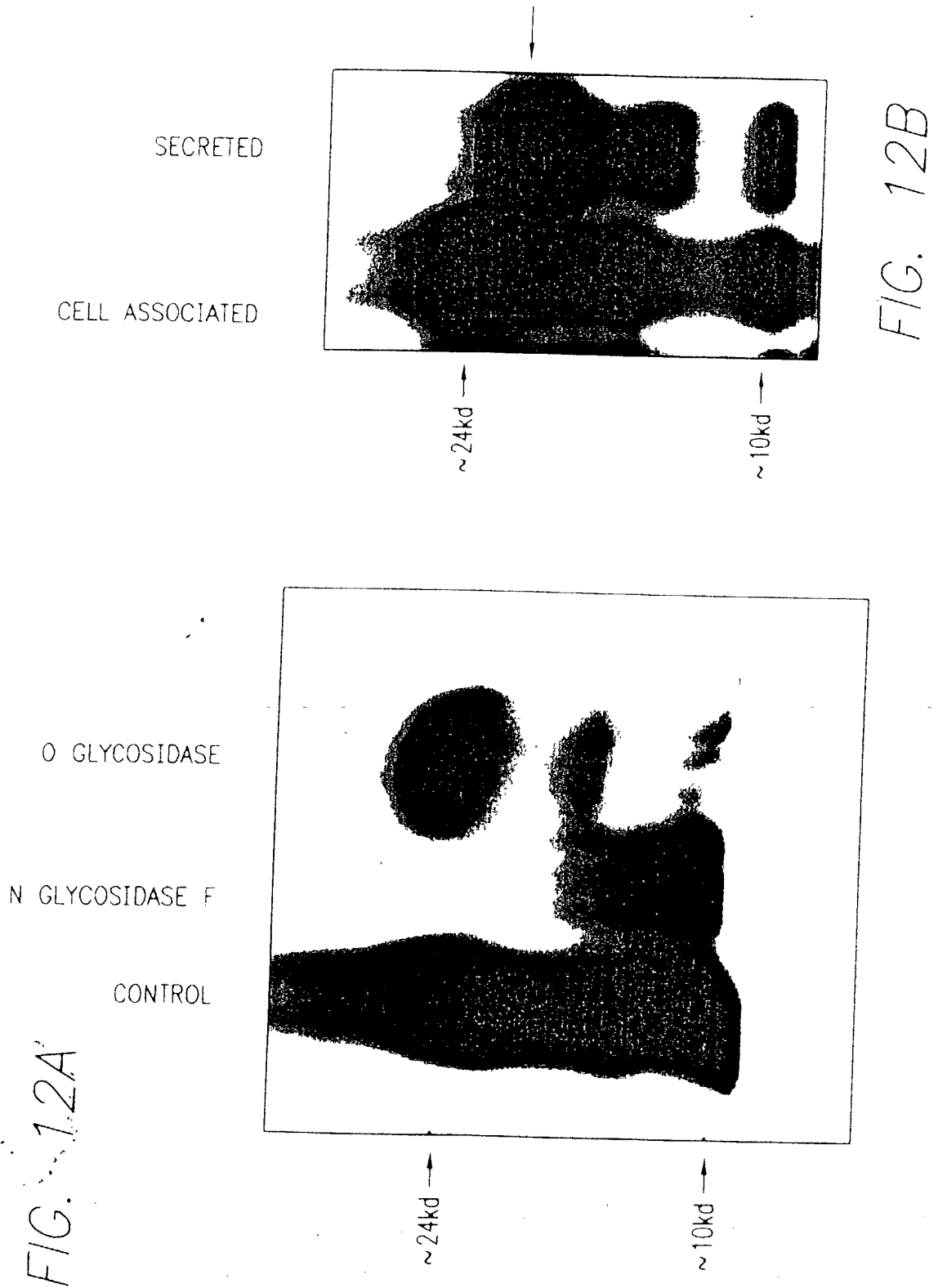


FIG. 11C



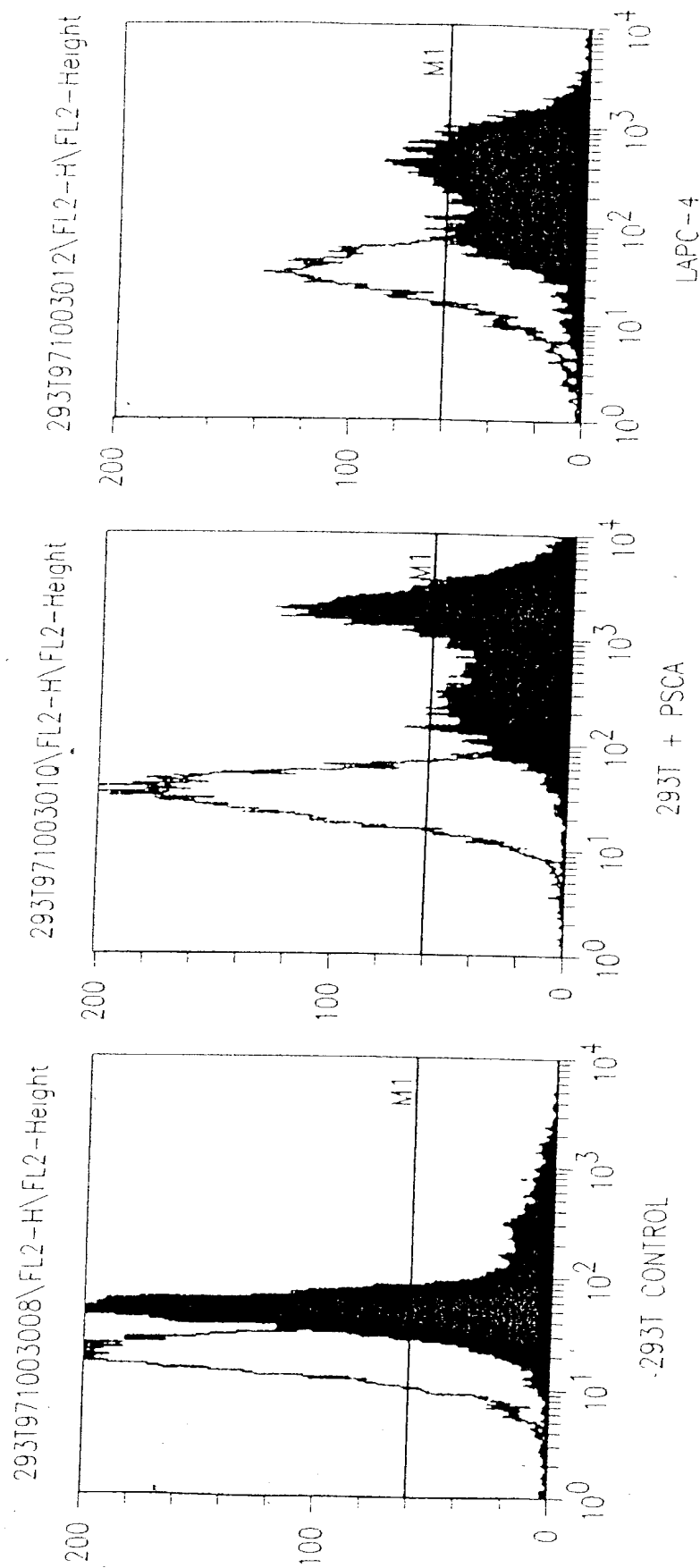


FIG. 12C

FIG. 13

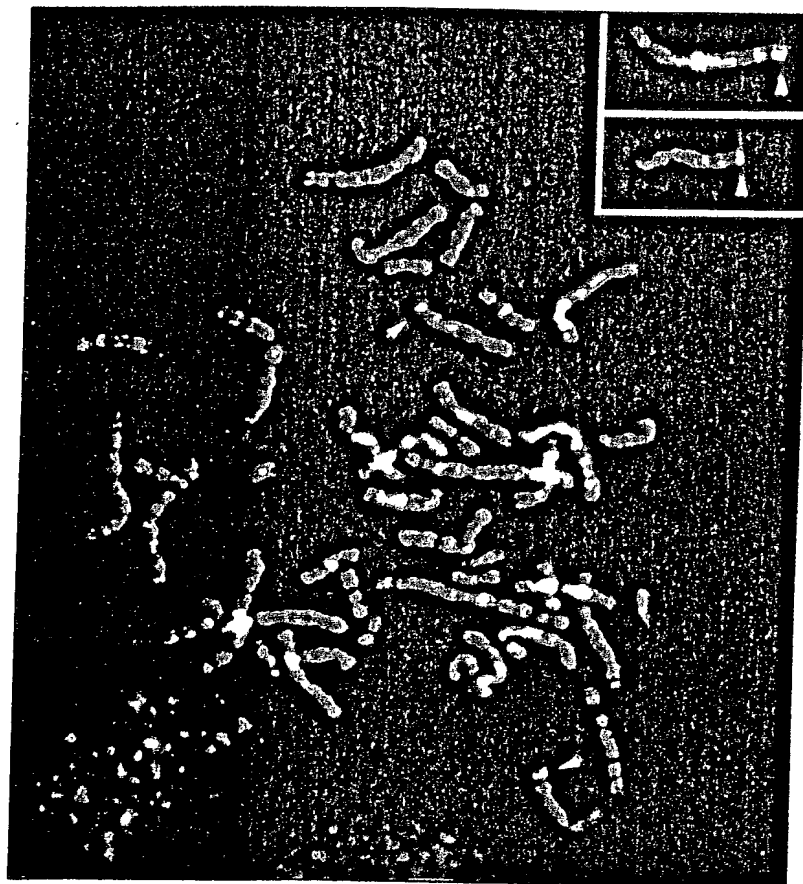


FIG. 14A

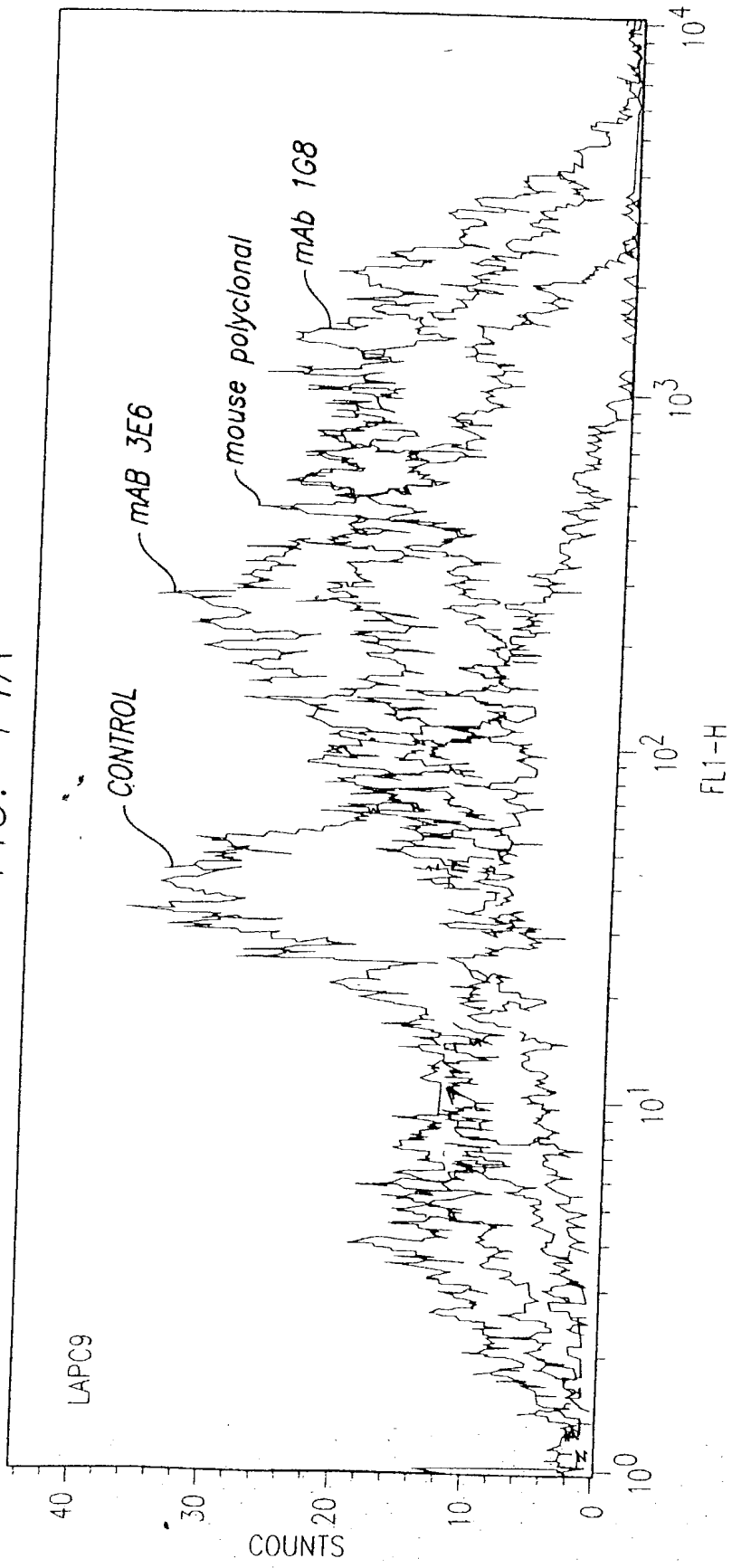


FIG. 14B

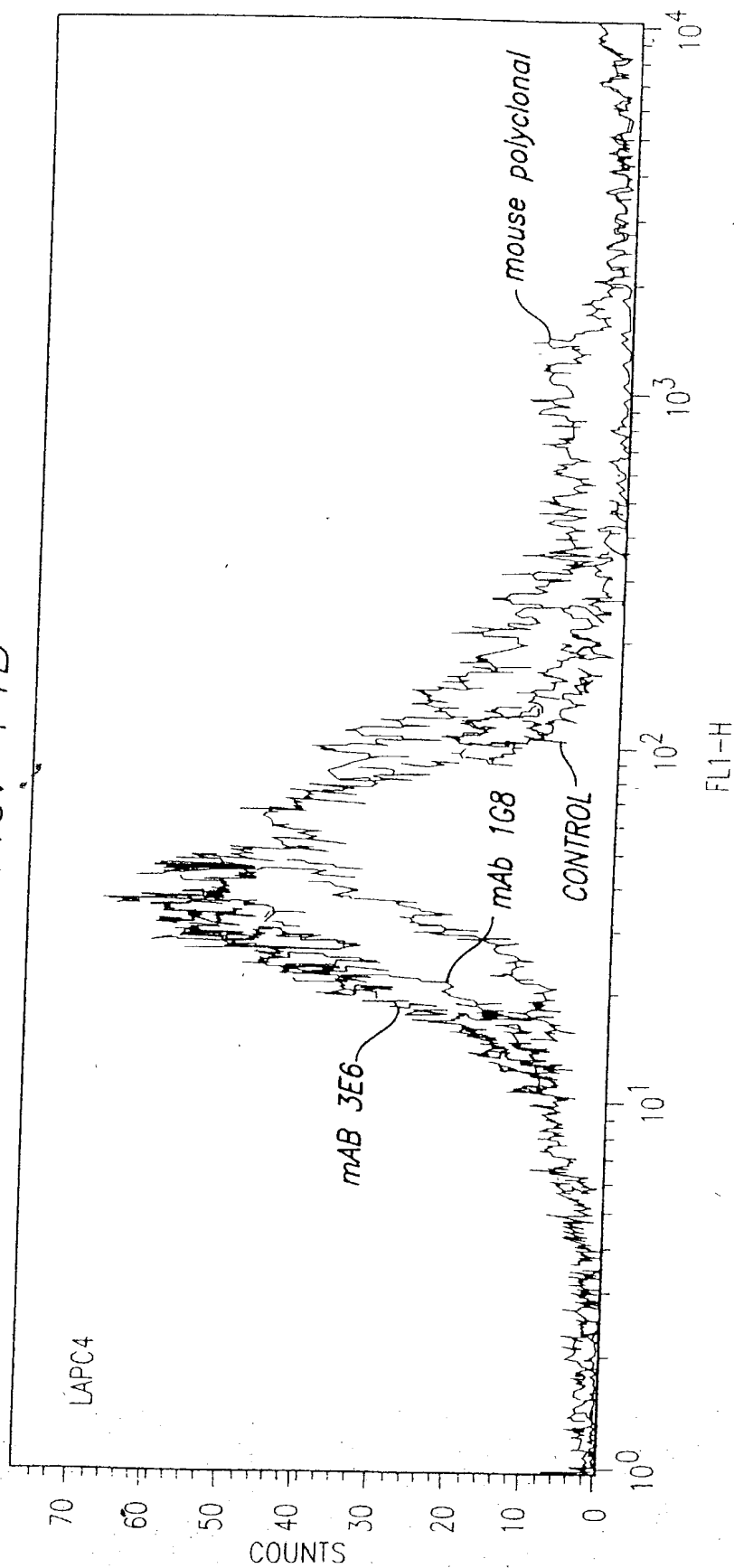


FIG. 14C

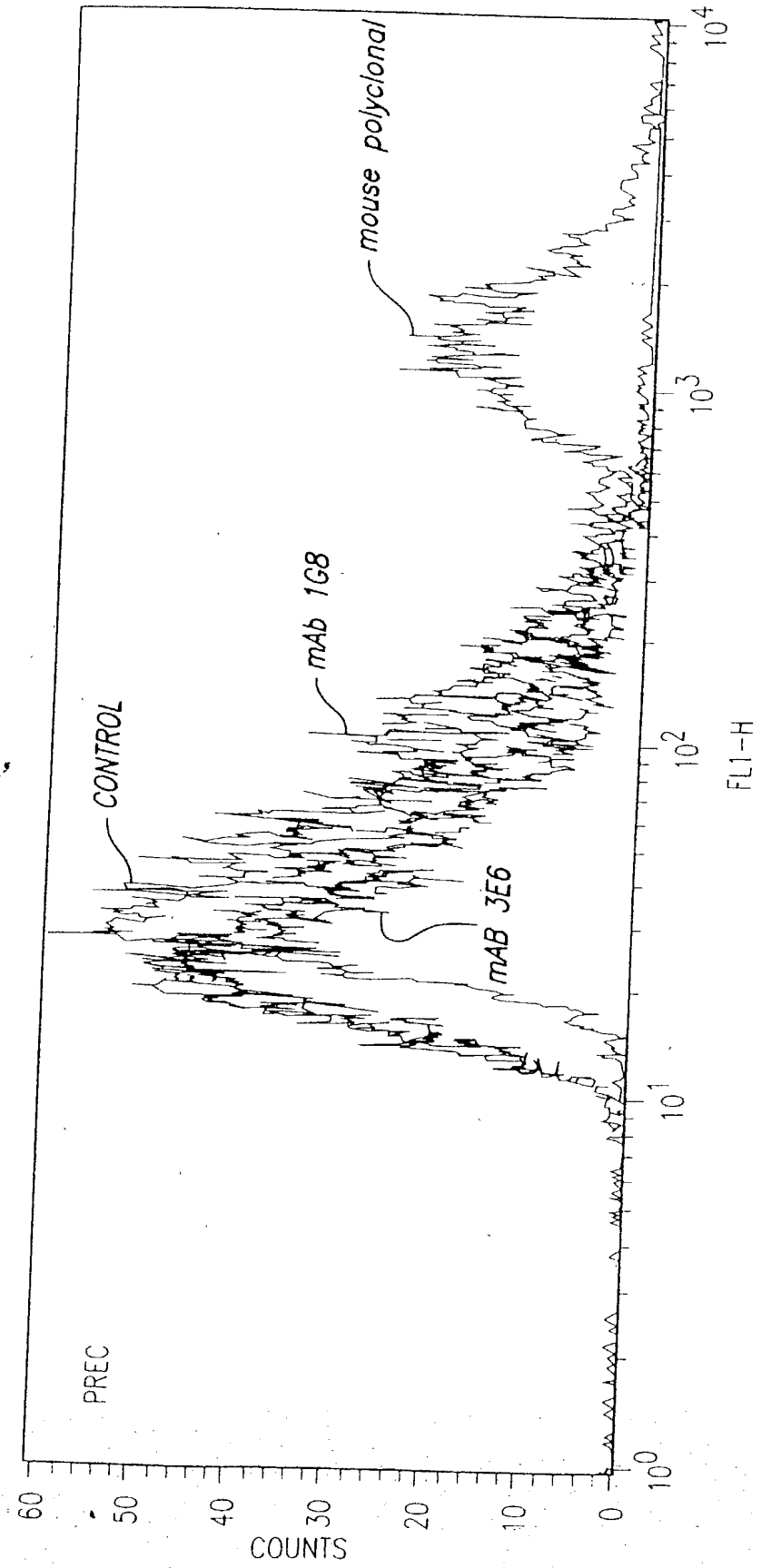


FIG. 15A

EPIIIOPE MAP

mAb	ISOIYPE	FL (18-98)	N (2-50)	M (46-109)	C (85-123)
1G8	IgG1 k	2.039	0.007	0.628	0.000
2H9	IgG1 k	1.318	0.863	0.032	0.021
3C5	IgG2a k	2.893	1.965	0.016	0.005
3E6	IgG3 k	0.328	0.024	0.069	0.370
4A10	IgG2a k	2.039	1.315	0.000	0.014
2A2	IgG2a k	1.366	0.733	0.010	0.003
3G3	IgG2a k	2.805	1.731	0.004	0.000

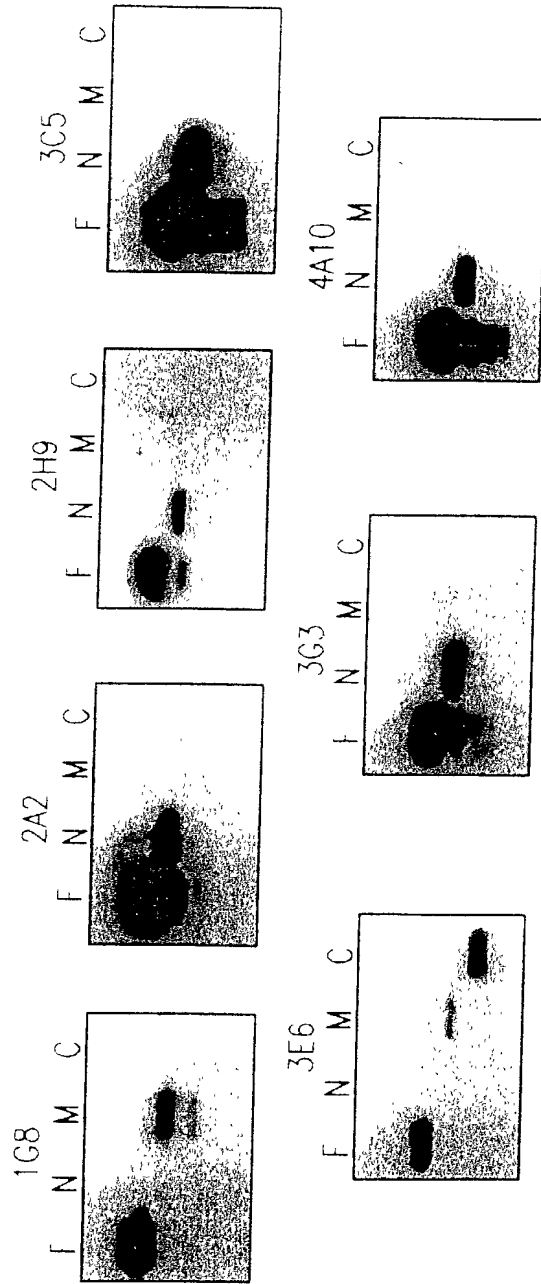


FIG. 15B

FIG. 16A

PROSTATE STEM CELL ANTIGEN (PSCA) IS A GPI-ANCHORED PROTEIN

1	M	K	I	F	L	P	V	L	A	A	L	L	G	V	E	R	A	S	S	hSCA-2
1	M	K	A	V	L	L	A	L	L	M	A	G	L	A	L	Q	P	G	T	hPSCA
1	M	K	T	V	L	F	L	L	A	T	Y	L	A	L	H	P	G	A	A	mPSCA
21	L	M	C	E	S	C	L	N	Q	K	S	N	L	Y	C	L	K	P	T	I
21	L	L	C	Y	S	C	K	A	Q	V	S	N	E	D	C	L	Q	V	E	N*
21	L	L	Q	C	Y	S	C	T	A	Q	M	N	N	R	D	C	L	N	V	Q
41	C	S	D	Q	D	N	Y	C	V	T	V	S	A	S	A	G	I	G	N	L
41	C	T	Q	L	G	E	Q	C	W	T	A	R	I	R	A	V	G	L	L	T
41	C	S	L	D	Q	H	S	C	F	T	S	R	I	R	A	I	C	L	V	T
61	V	T	F	G	H	S	L	S	K	T	C	S	P	A	C	P	I	P	E	G
61	V	-	-	-	-	-	-	I	S	X	C	C	S	L	N	C	V	D	D	S
61	V	-	-	-	-	-	-	I	S	X	C	C	S	L	N	C	V	D	D	S
81	V	N	V	G	V	A	S	M	G	T	S	C	C	Q	Q	S	F	E	C	N*
76	D	V	Y	V	G	K	K	-	N	I	T	C	C	G	D	T	D	L	C	N*
76	N	X	Y	L	C	K	K	-	N	I	T	C	C	G	Y	S	D	L	C	N*
101	S	A	A	D	G	G	L	R	A	S	V	T	L	L	G	A	G	L	L	L
95	S	G	A	H	A	L	Q	P	A	A	A	I	L	A	L	P	A	L	G	G
95	N	G	A	H	T	L	K	P	P	T	T	L	G	L	L	T	V	L	C	S
121	S	L	L	P	A	L	L	R	F	G	P	-	-	-	-	-	-	-	-	-
115	L	L	L	W	G	P	G	Q	L	-	-	-	-	-	-	-	-	-	-	-
115	L	L	L	W	G	S	S	R	L	-	-	-	-	-	-	-	-	-	-	-

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PROSTATE STEM CELL ANTIGEN (PSCA) IS A GPI-ANCHORED PROTEIN

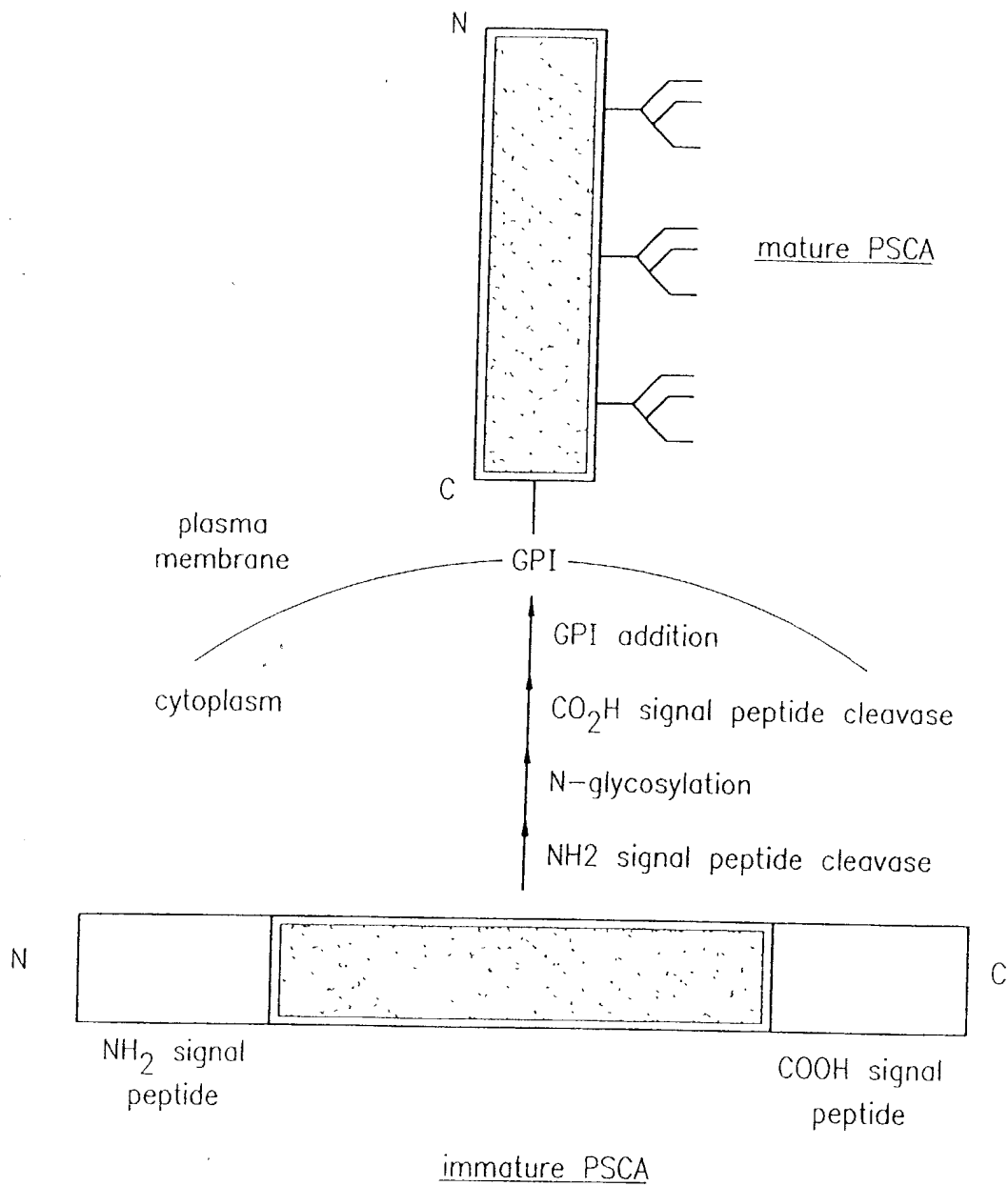
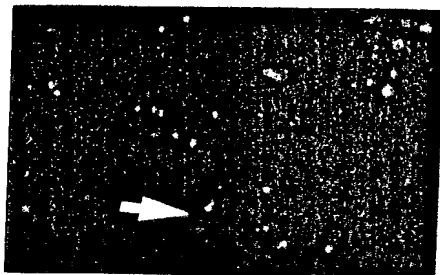


FIG. 16B

FIG. 17

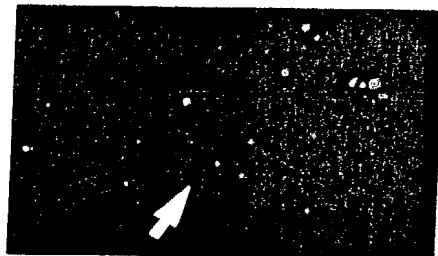
FISH ANALYSIS OF PSCA AND c-myc IN PROSTATE CANCER

GAIN CHROMOSOME 8

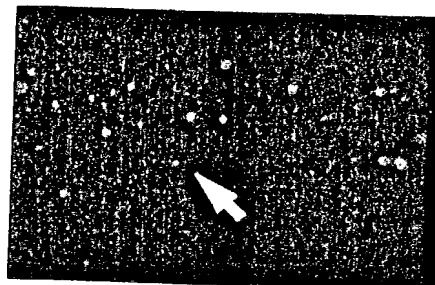


#34 c-myc

#34 PSCA



AMPLIFICATION



#75 c-myc

#75 PSCA

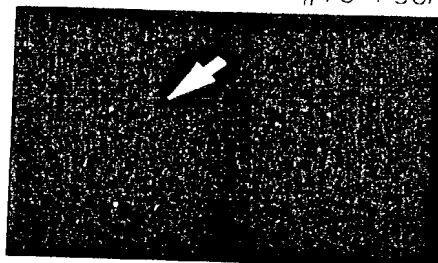
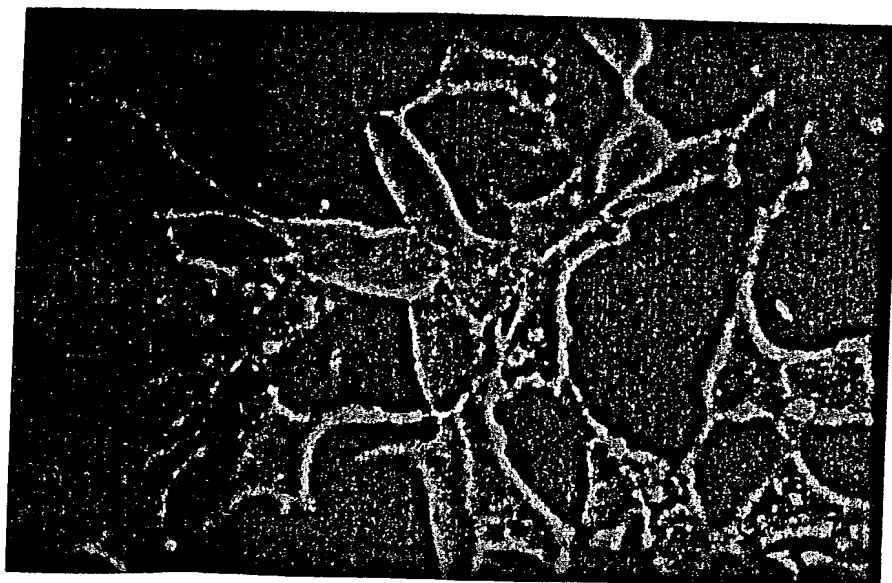


FIG. 18



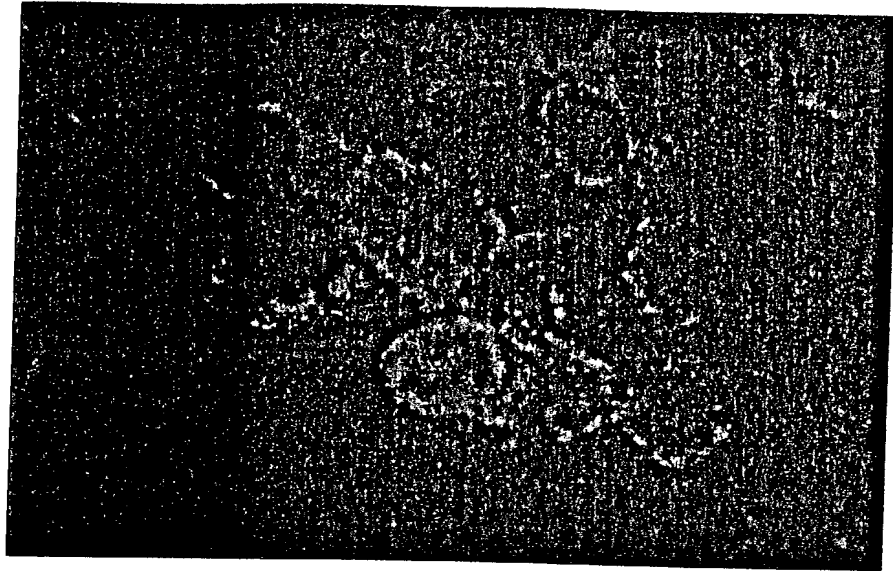


FIG. 19

FIG. 20

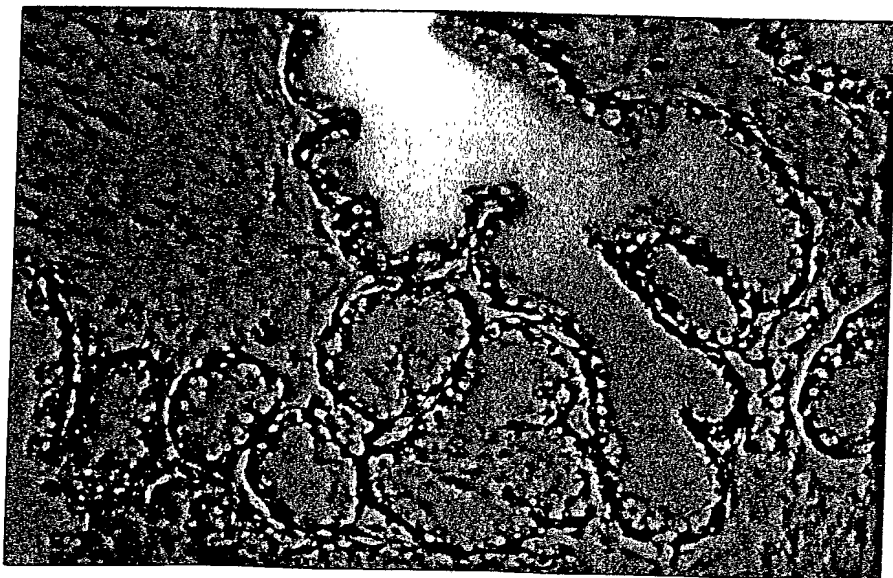
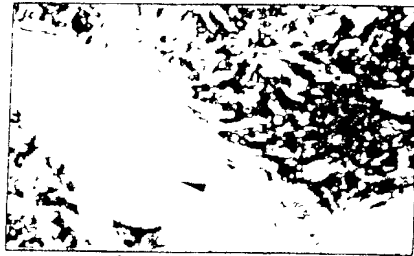


FIG. 21

PSCA IMMUNOSTAINING OF PRIMARY TUMORS



patient 1 mAb 1G8



patient 2: mAb 1G8



patient 3: mAb 1G8



patient 4: mAb 3E6

FIG. 22

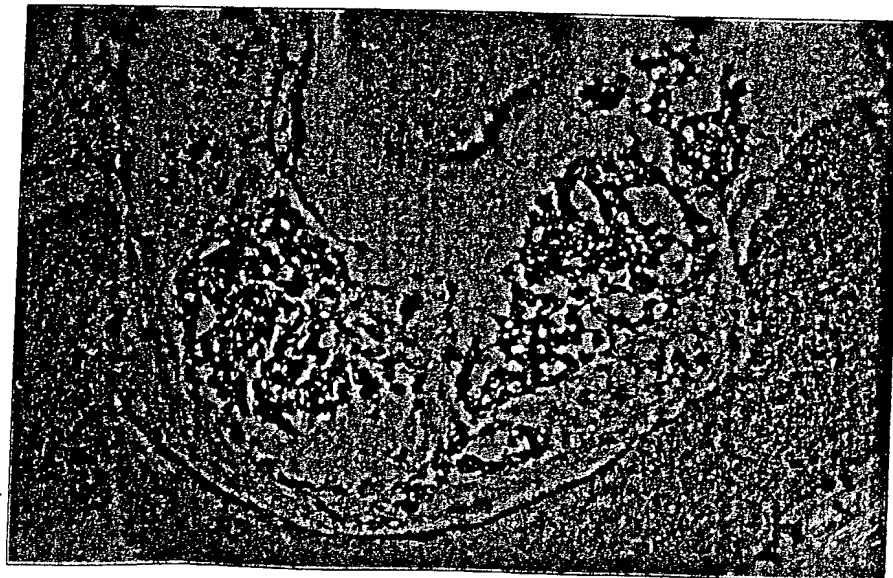




FIG. 23

FIG. 24

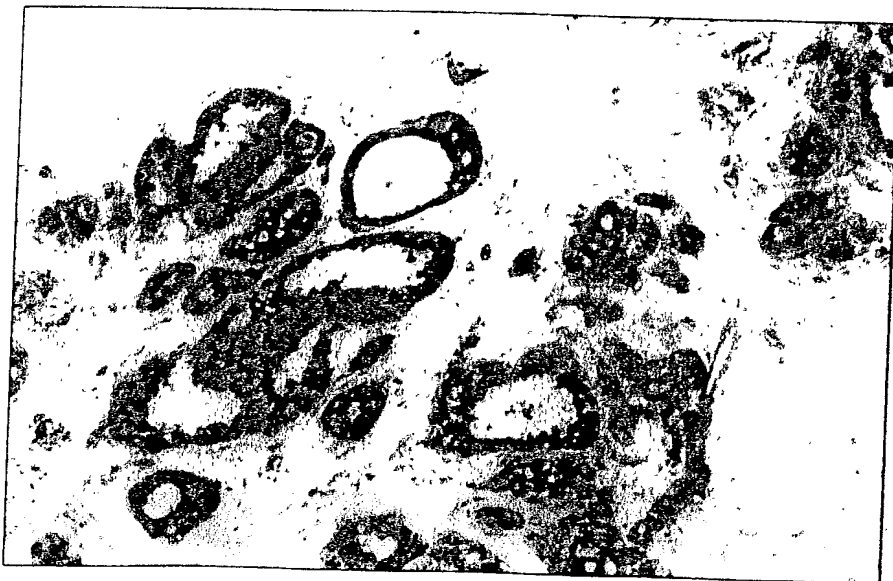
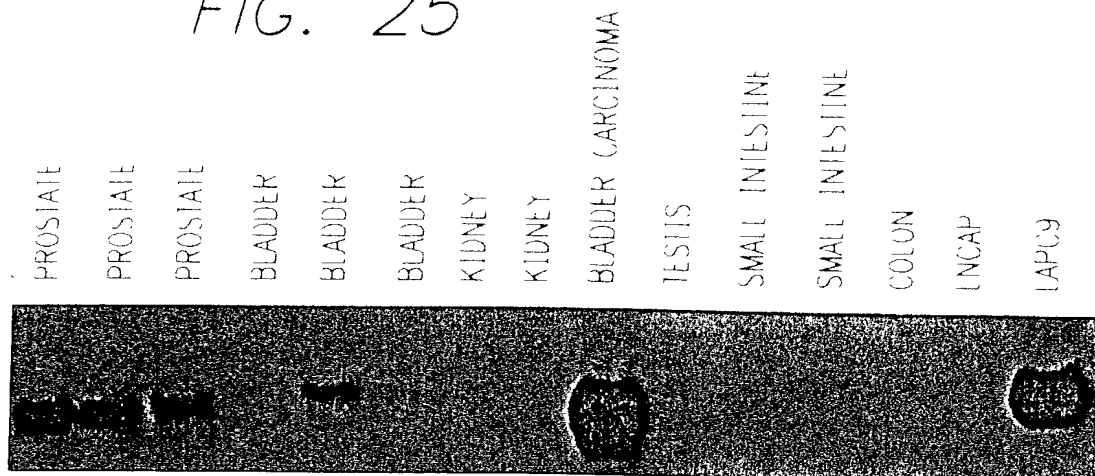


FIG. 25



PSCA NORTHERN



FIG. 26

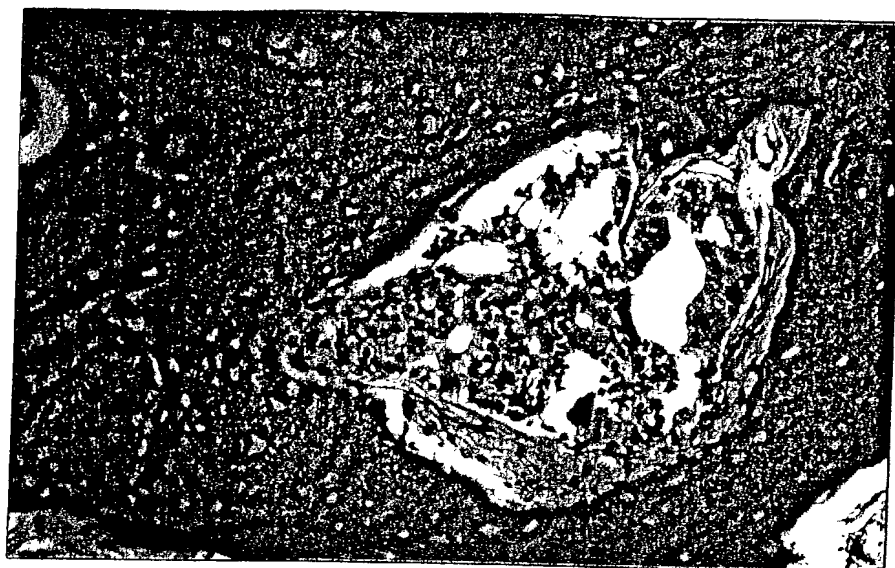
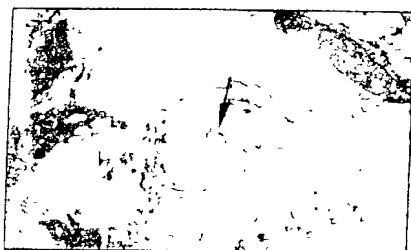


FIG. 27

PSCA IMMUNOSTAINING OF BONY METASTASES



Patient 5: H and E
and mAb 1G8



Patient 4: H and E
and mAb 3E6

FIG. 28

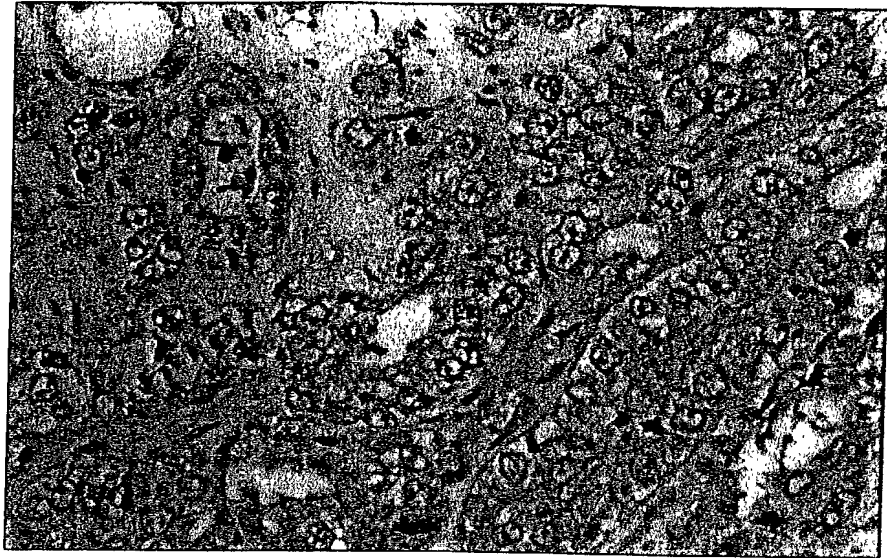
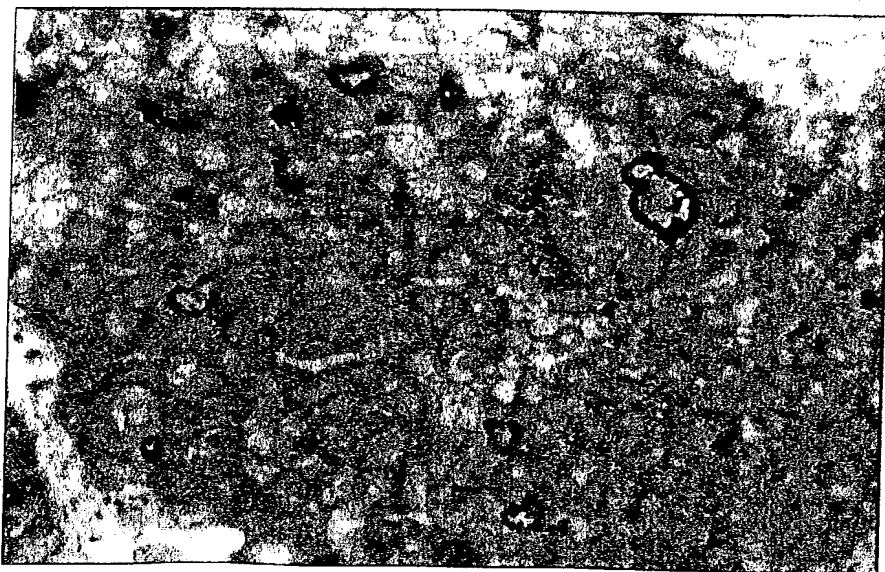


FIG. 29

FIG. 30



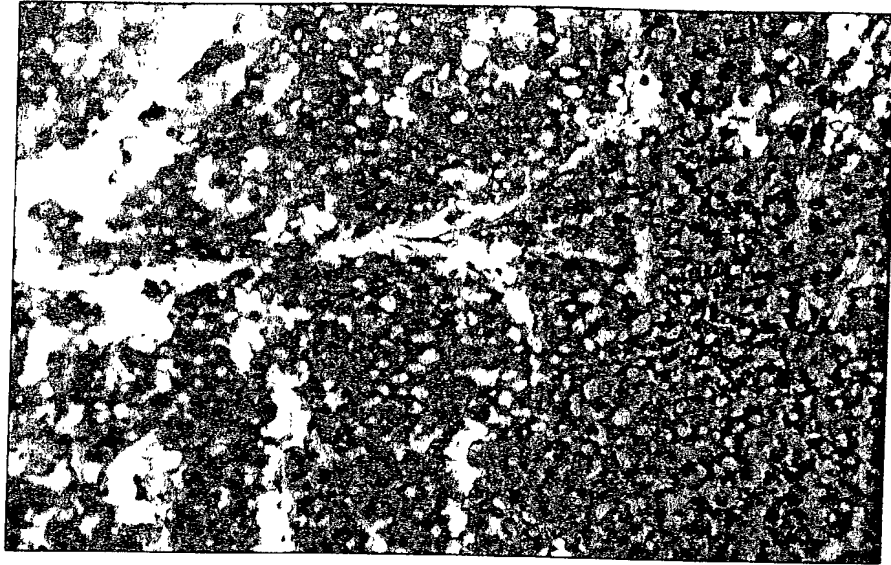


FIG. 31

FIG. 32

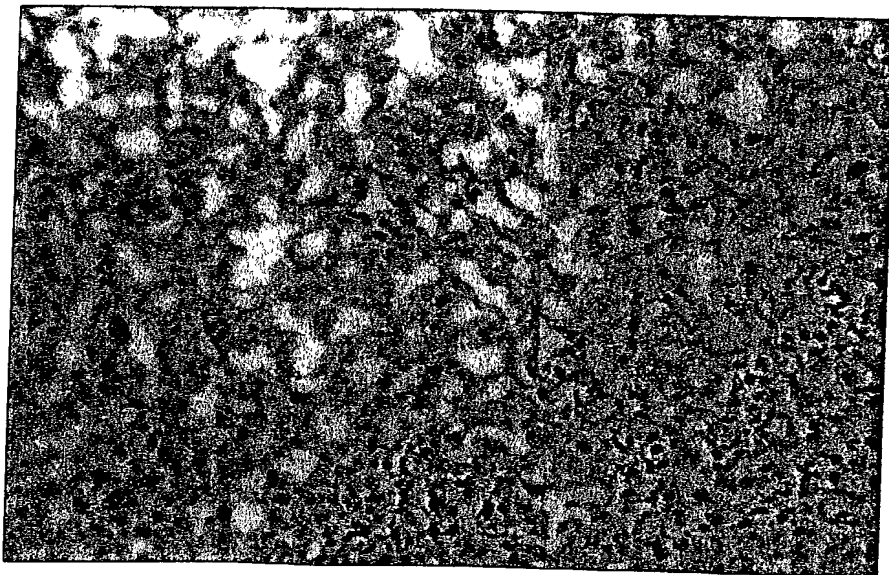
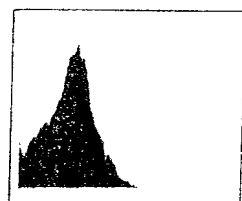


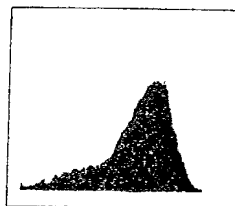
FIG. 33

PSCA EXPRESSION IN LAPC-9 XENOGRFT BY FACS

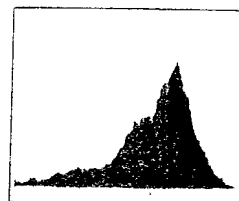
SECONDARY ANTIBODY



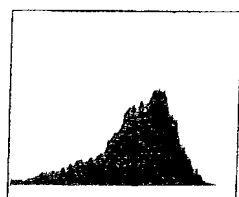
1G8



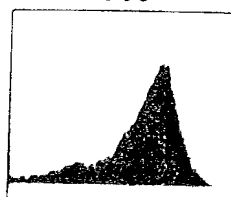
2H9



4A10



3C5



3E6

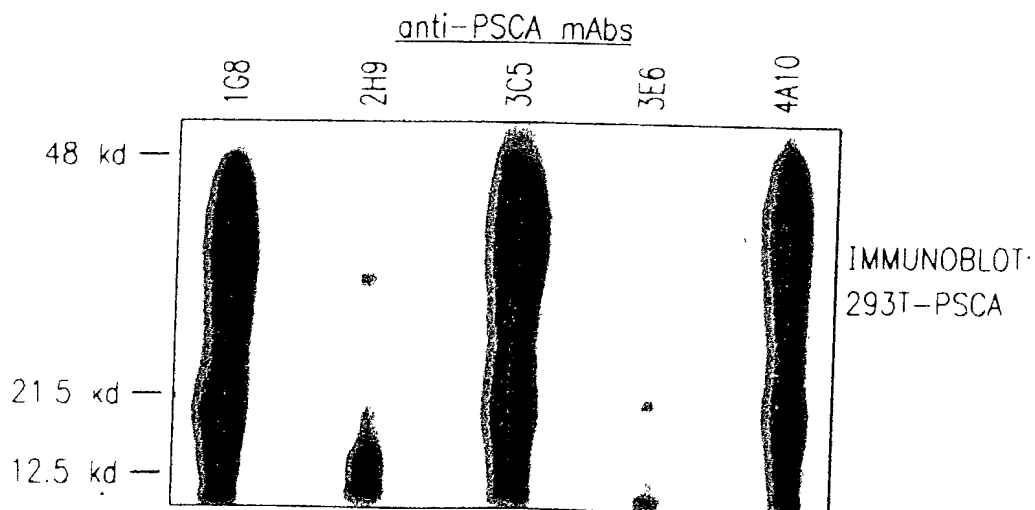
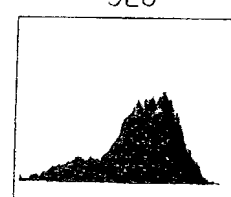


FIG. 34

FIG. 35

IMMUNOFLUORESCENT STAINING OF LNCaP-PSCA CELLS

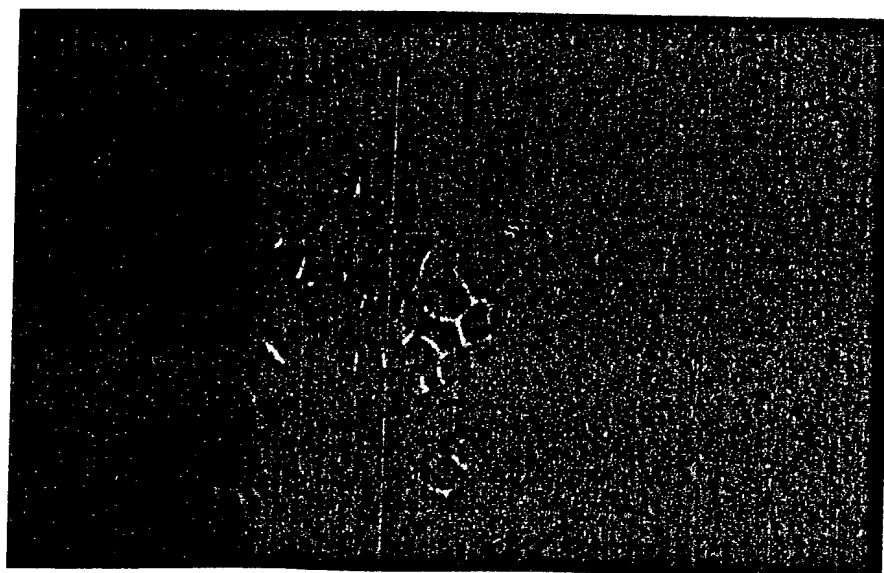
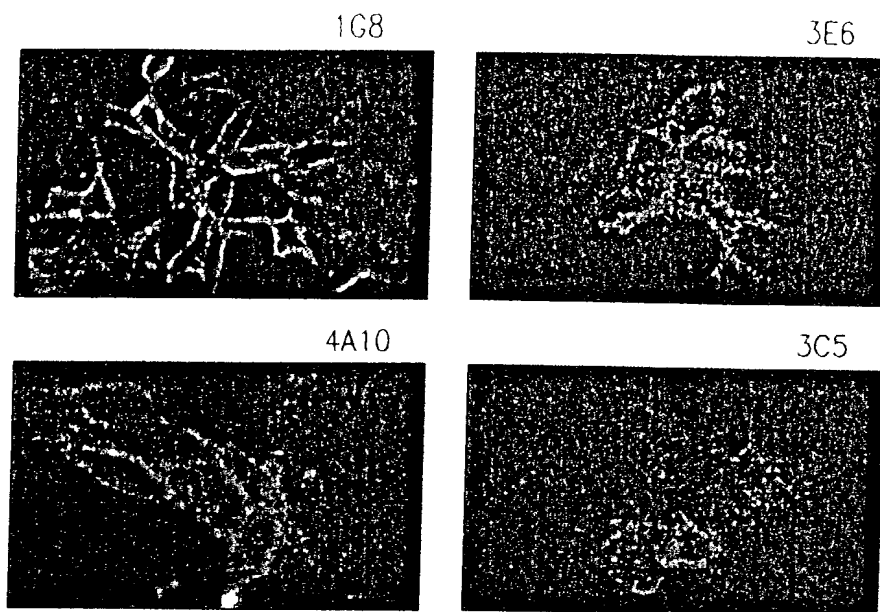


FIG. 36

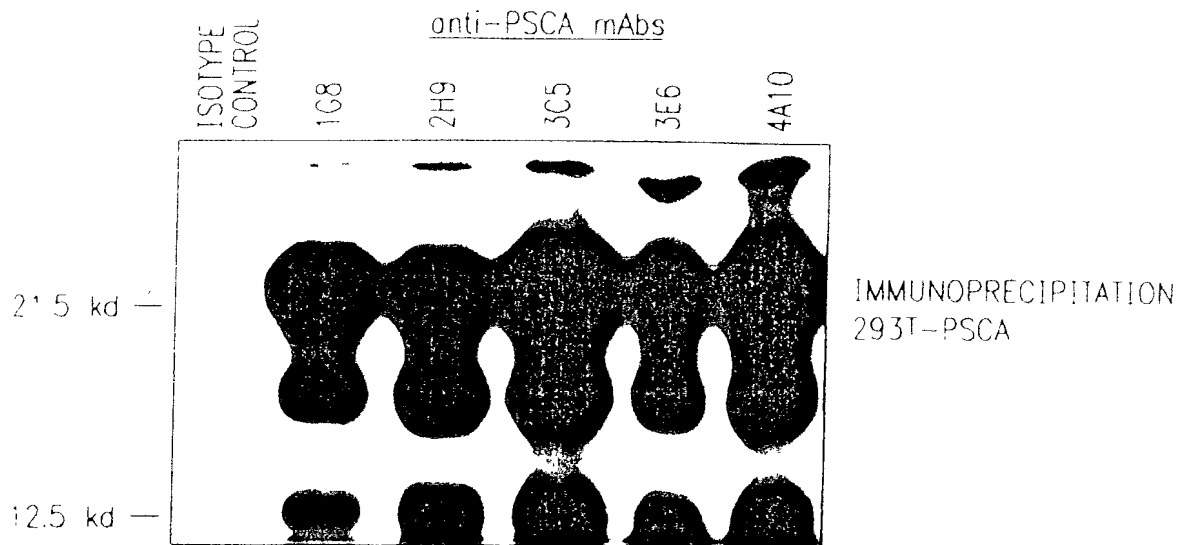


FIG. 37

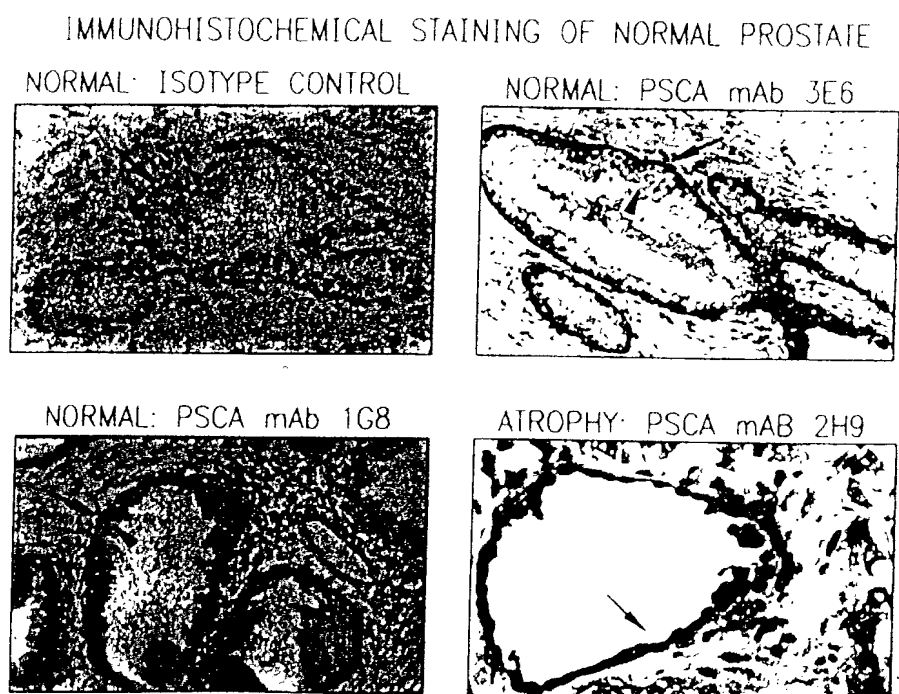
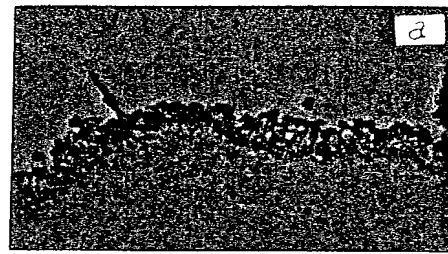
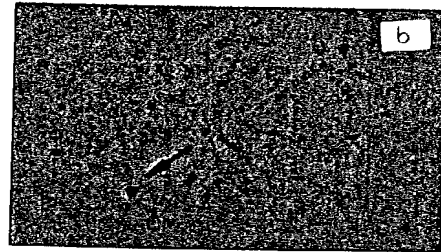


FIG. 38

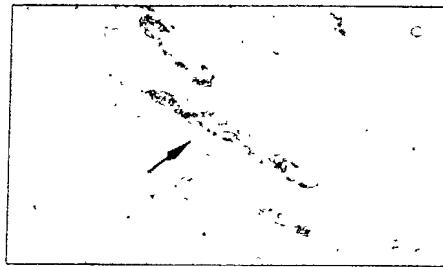
FIG. 39A



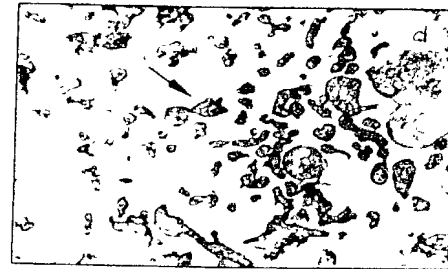
BLADDER: 1G8



COLON: 1G8



KIDNEY 3E6



PLACENTA: 3E6

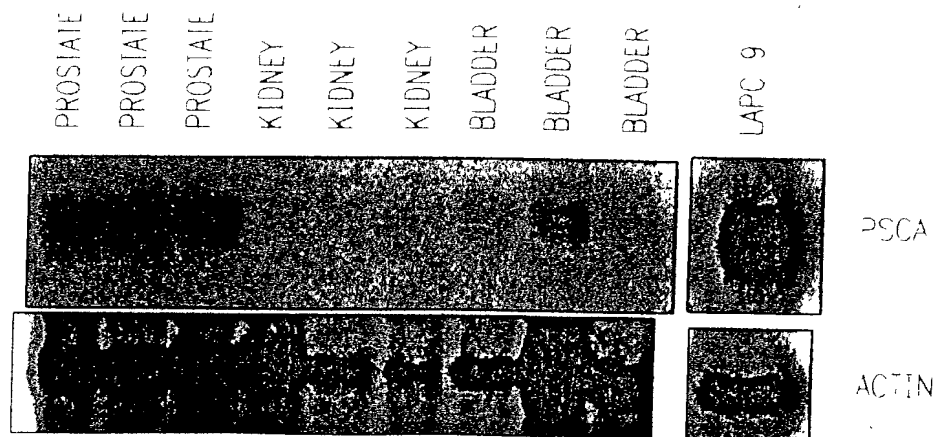


FIG. 39B

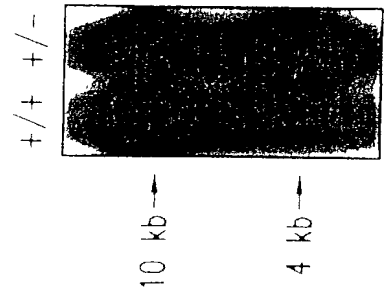
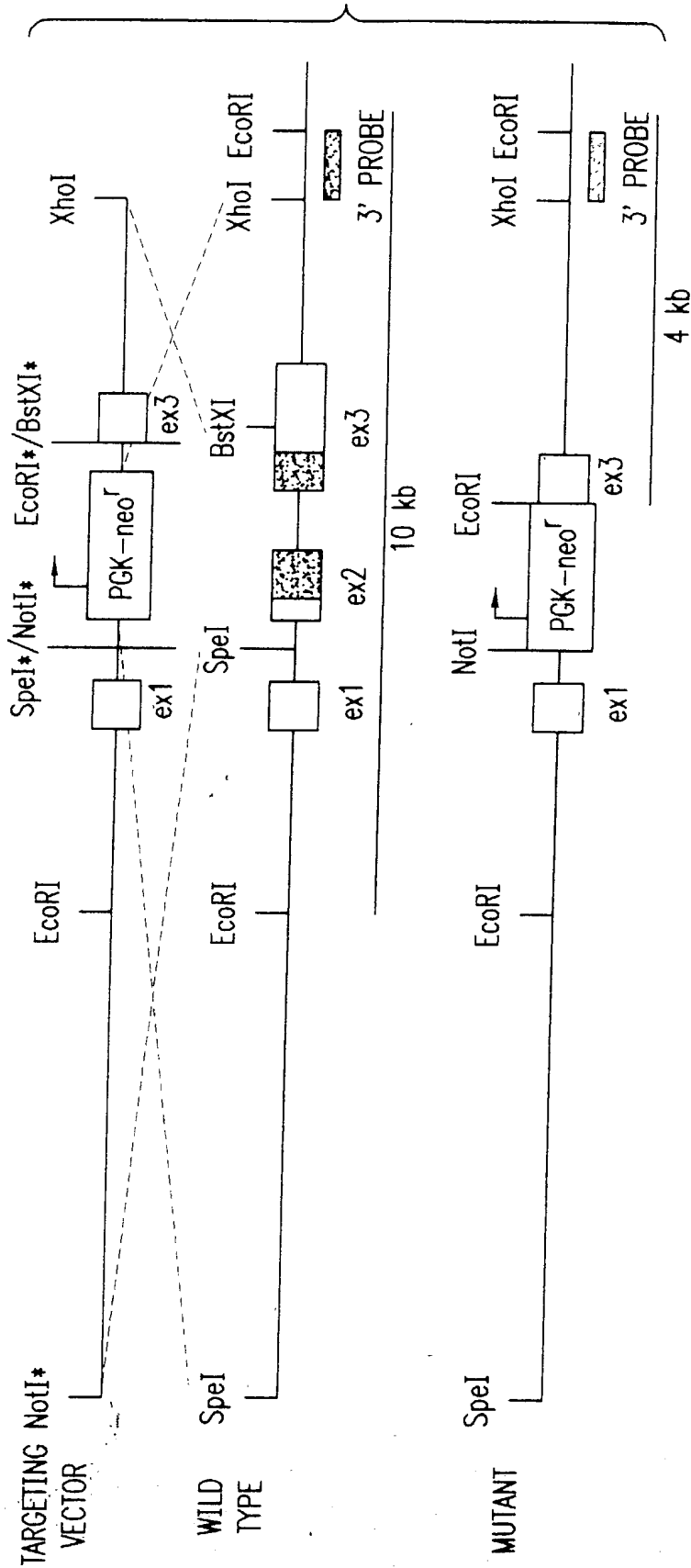


FIG. 40B

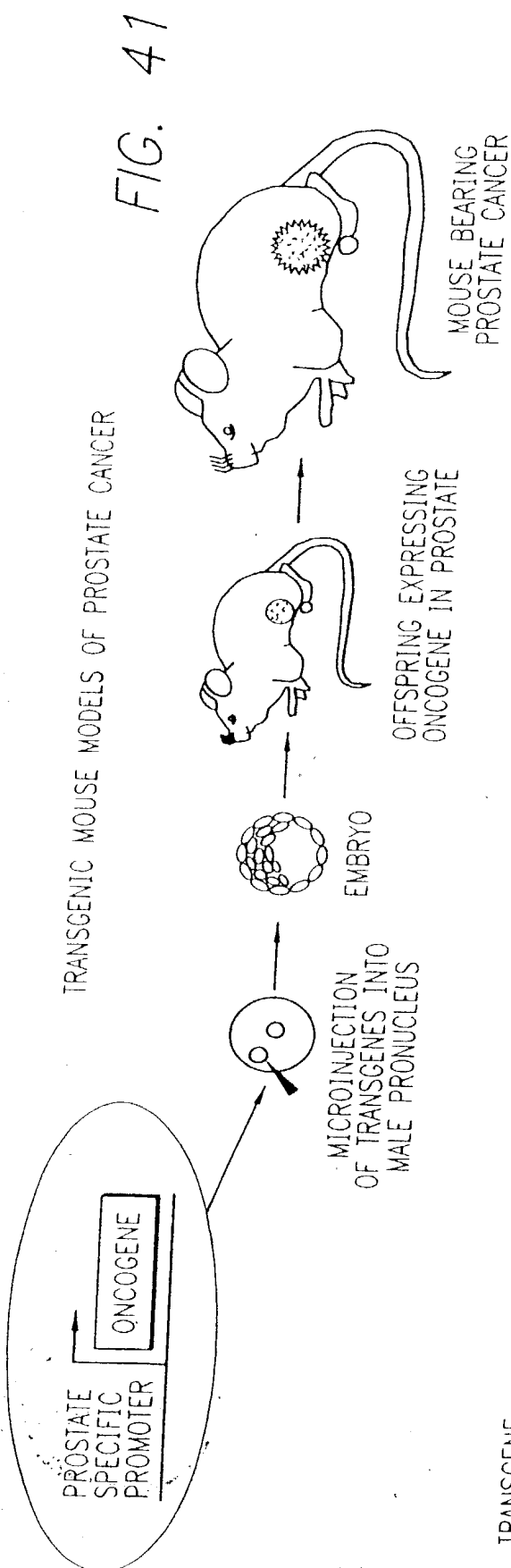


FIG. 41

TRANSGENIC MOUSE MODELS OF PROSTATE CANCER

TRANSGENE	TARGET TISSUES	CHARACTERISTICS
C3(1) (-3 kb)/ SV40 LARGE+SMALL, T MAROULAKOU et al. 1994 PNAS	PROSTATE (SECRETORY CELLS) URETHRAL, MAMMARY AND SWEAT GLAND	LOW-GRADE PIN 8-12 WKS HIGH-GRADE PIN 8-12 WKS INVASIVE CARCINOMA 28 WKS NO METASTASES
PROBASIN (-426 bp)/ SV40 LARGE+SMALL, T GREENBERG et al. 1995 PNAS	PROSTATE (SECRETORY CELLS)	LOW-GRADE PIN 5-8 WKS HIGH-GRADE PIN 8-12 WKS INVASIVE CARCINOMA 12 WKS METASTASES IN LYMPH NODE, LUNG, LIVER AND BONE
CRYPTIDIN2 (-6.5 kb)/ SV40 LARGE+SMALL, T GARABEDIAN et al. 1998 PNAS	PROSTATE (NEUROENDOCRINE CELLS) SMALL INTESTINE	LOW-GRADE PIN 8-12 WKS HIGH-GRADE PIN 8-12 WKS INVASIVE CARCINOMA 16 WKS METASTASES IN LYMPH NODE, LUNG, LIVER, AND BONE

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093046Z APR 68 100000Z



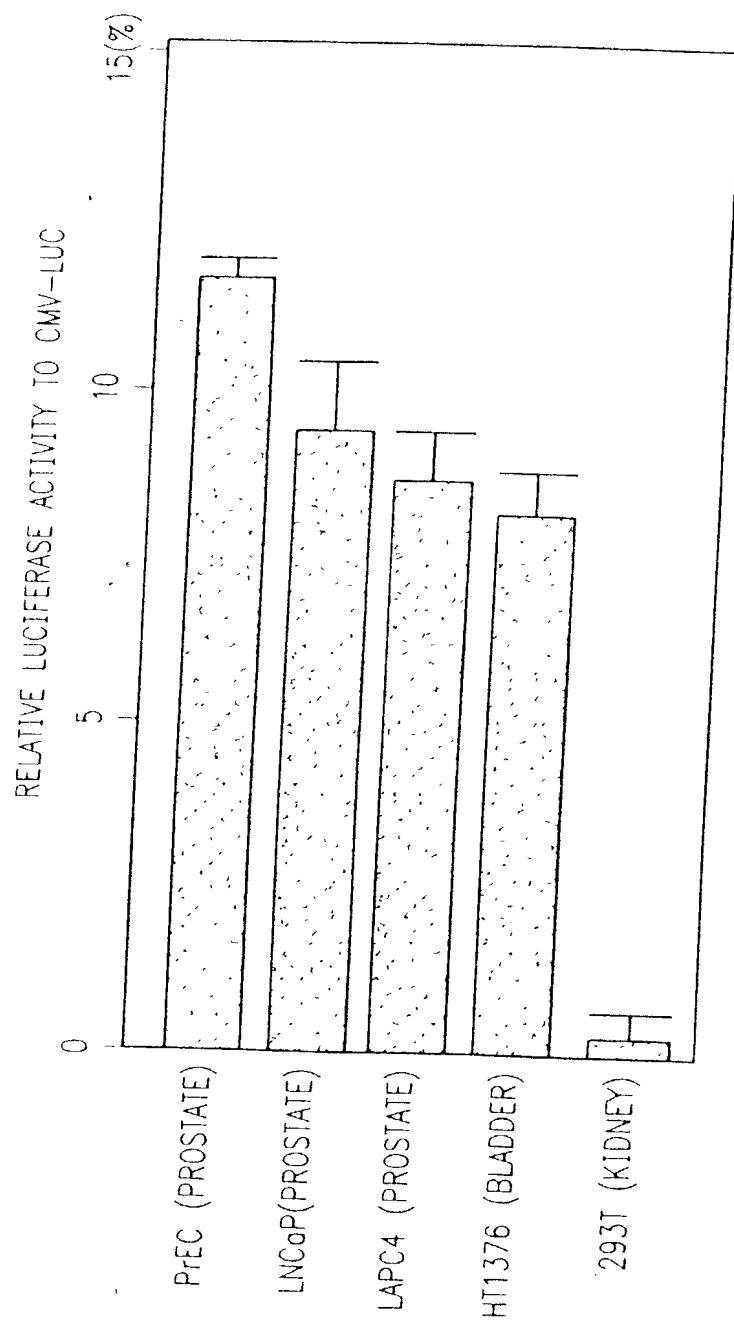


FIG. 43

IDENTIFICATION OF PROSTATE-SPECIFIC ELEMENTS WITHIN PSCA PROMOTER SEQUENCES

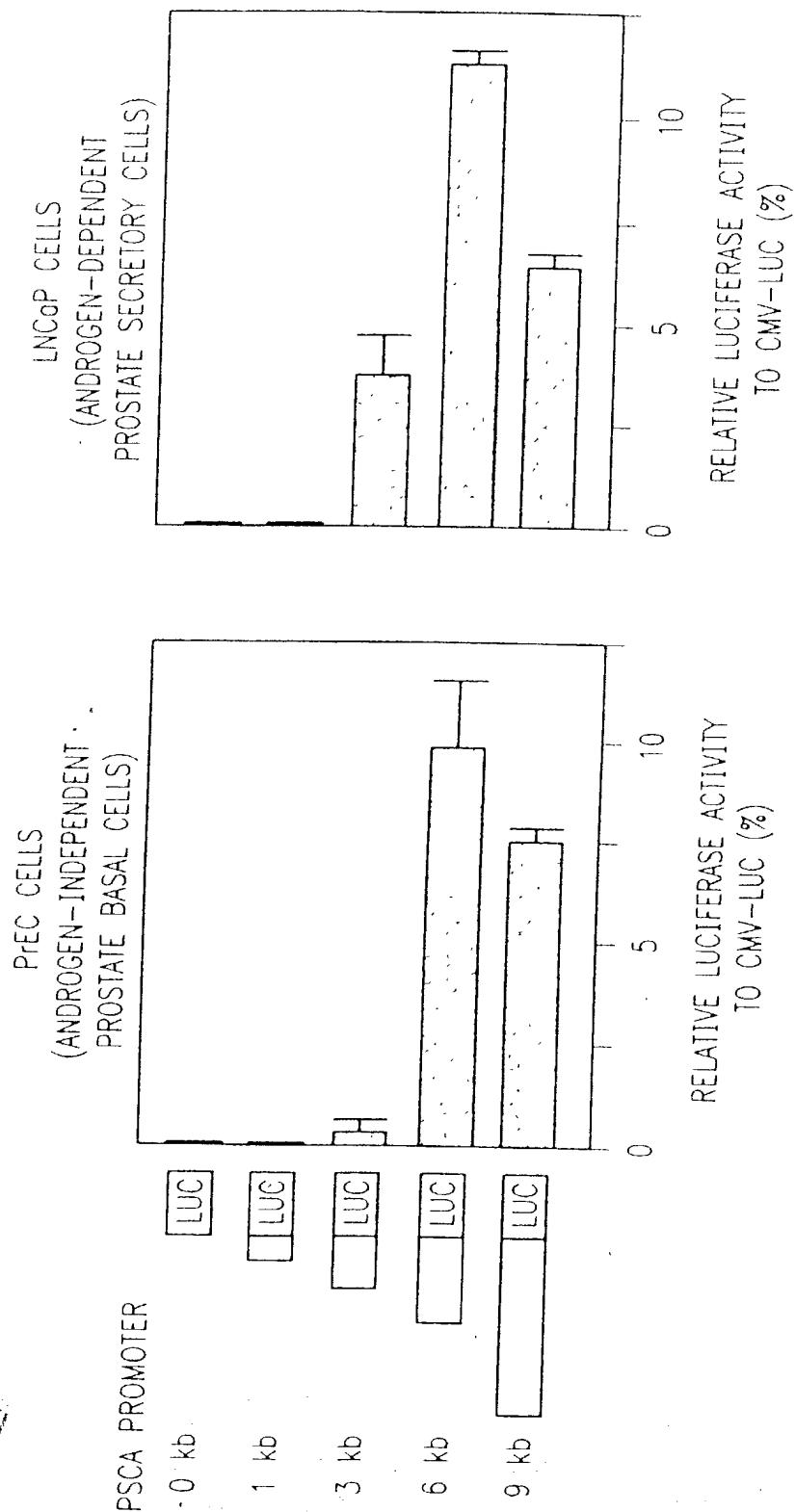
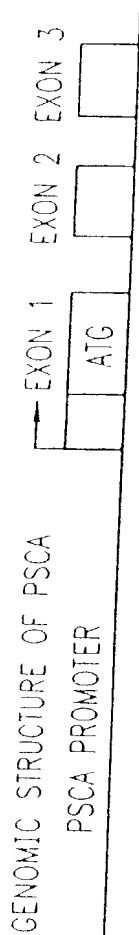


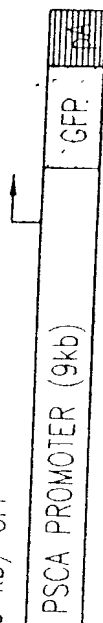
FIG. 44

FIG. 45

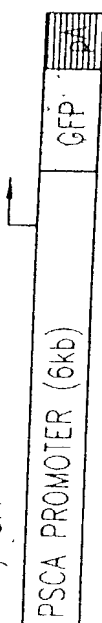
UPDATE OF TRANSGENIC MOUSE PROJECTS



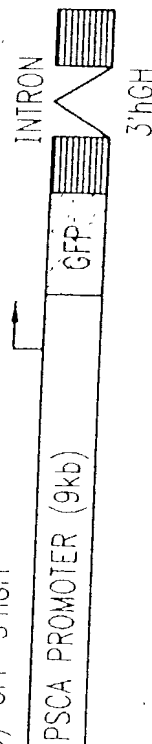
PSCA(9 kb)-GFP



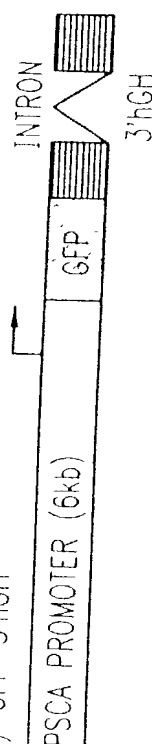
PSCA(6 kb)-GFP



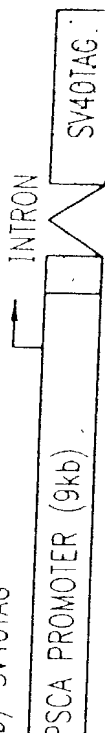
PSCA(9 kb)-GFP-3'hGH



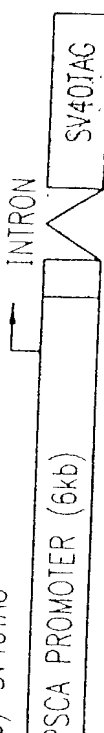
PSCA(6 kb)-GFP-3'hGH



PSCA(9 kb)-SV40TAG



PSCA(6 kb)-SV40TAG



NUMBER OF FOUNDERS (DNA POSITIVE)
2
1
6
8
3
9

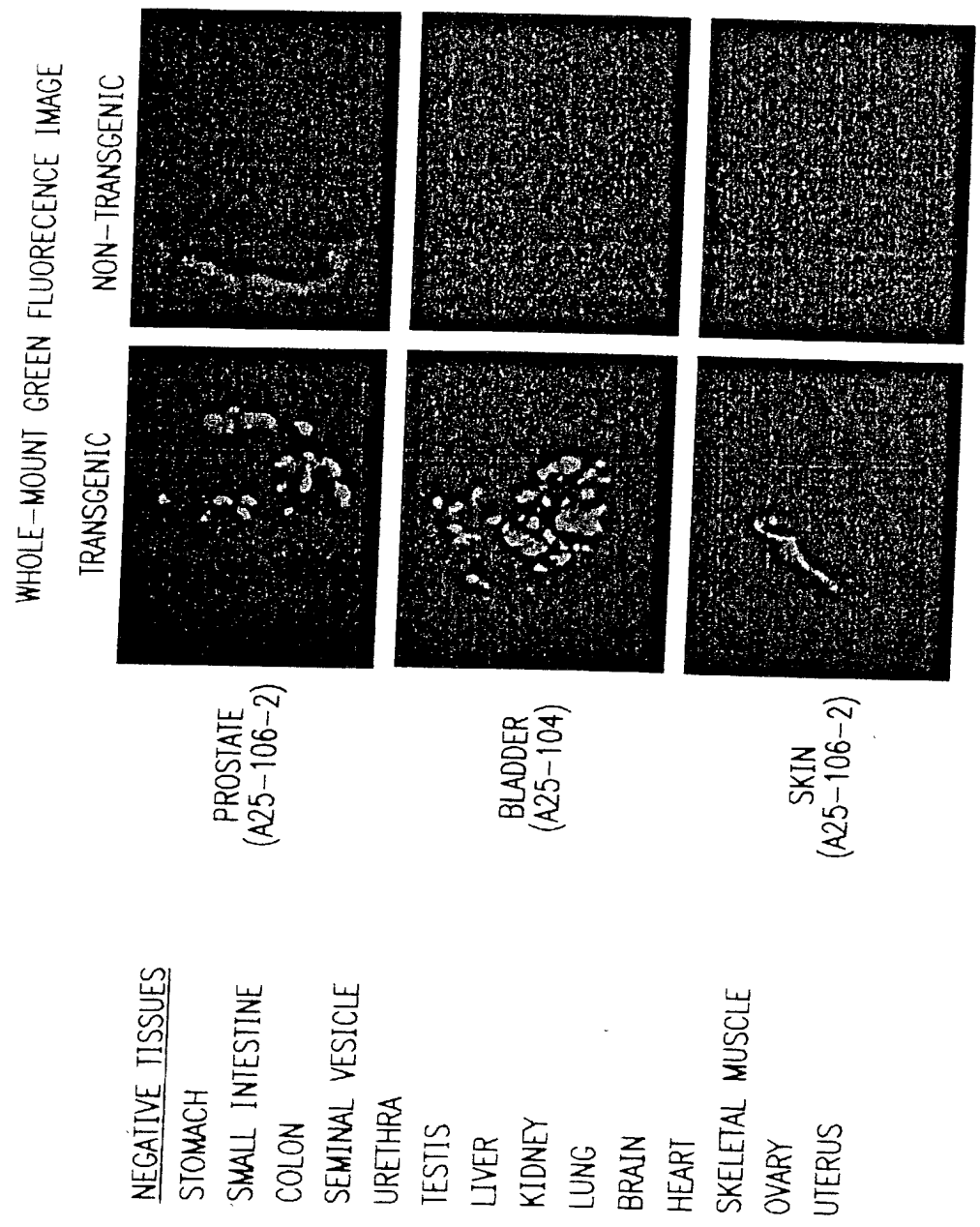


FIG. 46

- NEGATIVE TISSUES
- STOMACH
 - SMALL INTESTINE
 - COLON
 - SEMINAL VESICLE
 - URETHRA
 - TESTIS
 - LIVER
 - KIDNEY
 - LUNG
 - BRAIN
 - HEART
 - SKELETAL MUSCLE
 - OVARY
 - UTERUS

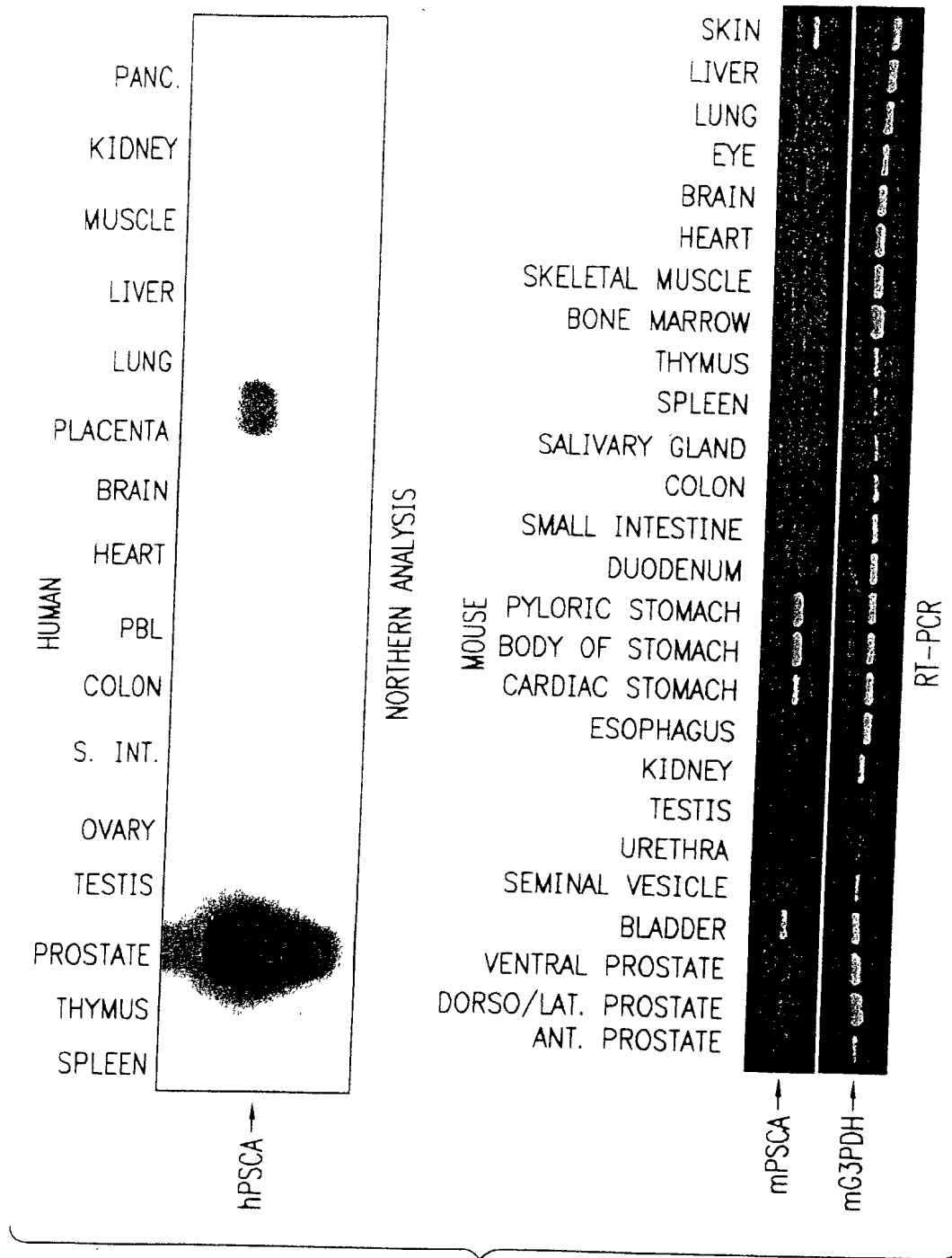
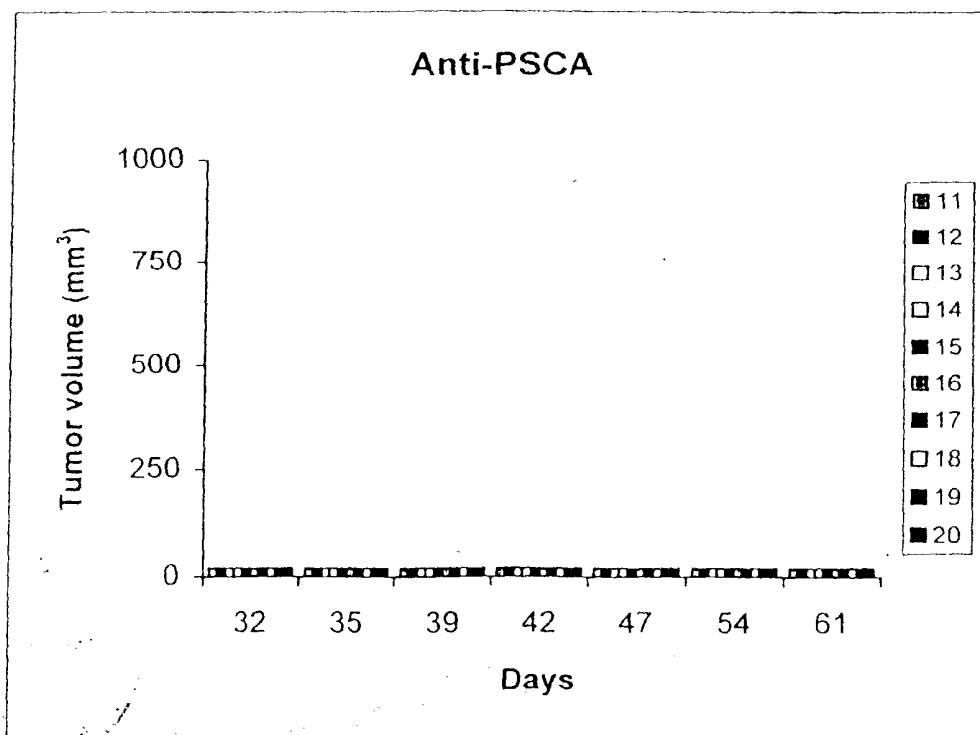
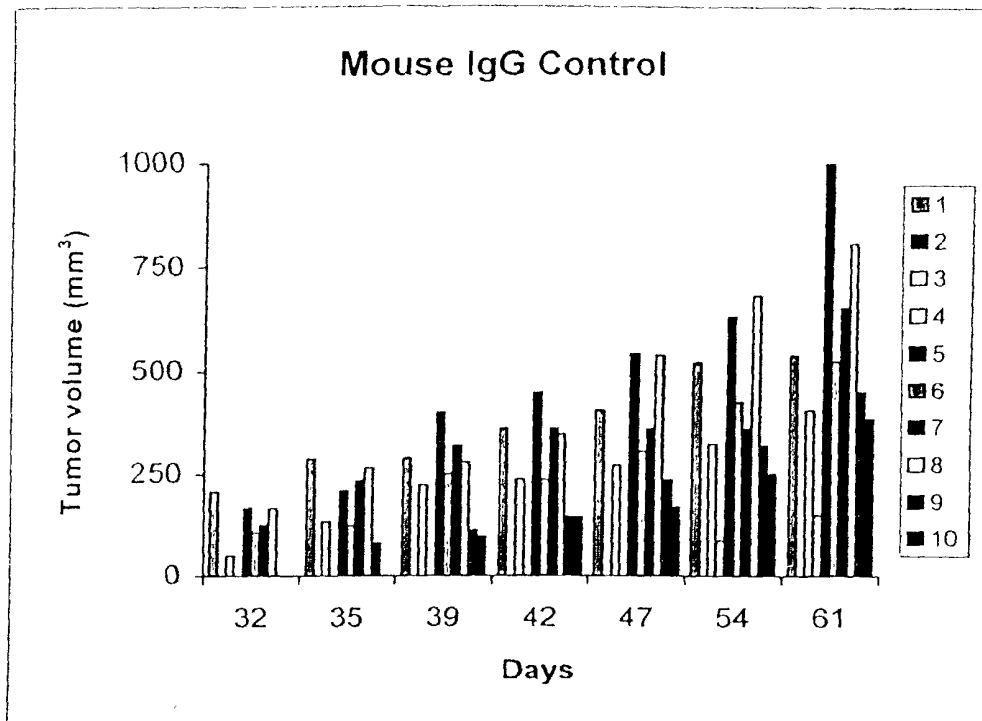


FIG. 47



A

FIG. 49

Epitope recognized (OD 450 nm)

mAb	Isotype	F (18-98)	N (2-50)	M (46-109)	C (85-123)
1G8	IgG1 k	1.485	0.004	1.273	0.003
2A2	IgG2a k	0.973	0.631	0.023	0.010
2H9	IgG1 k	1.069	1.026	0.002	0.001
3C5	IgG2a k	1.916	1.709	0.006	0.002
3E6	IgG3 k	1.609	0.036	1.133	2.118
3G3	IgG2a k	2.805	1.731	0.004	0.000
4A10	IgG2a k	1.053	0.493	0.000	0.001

B

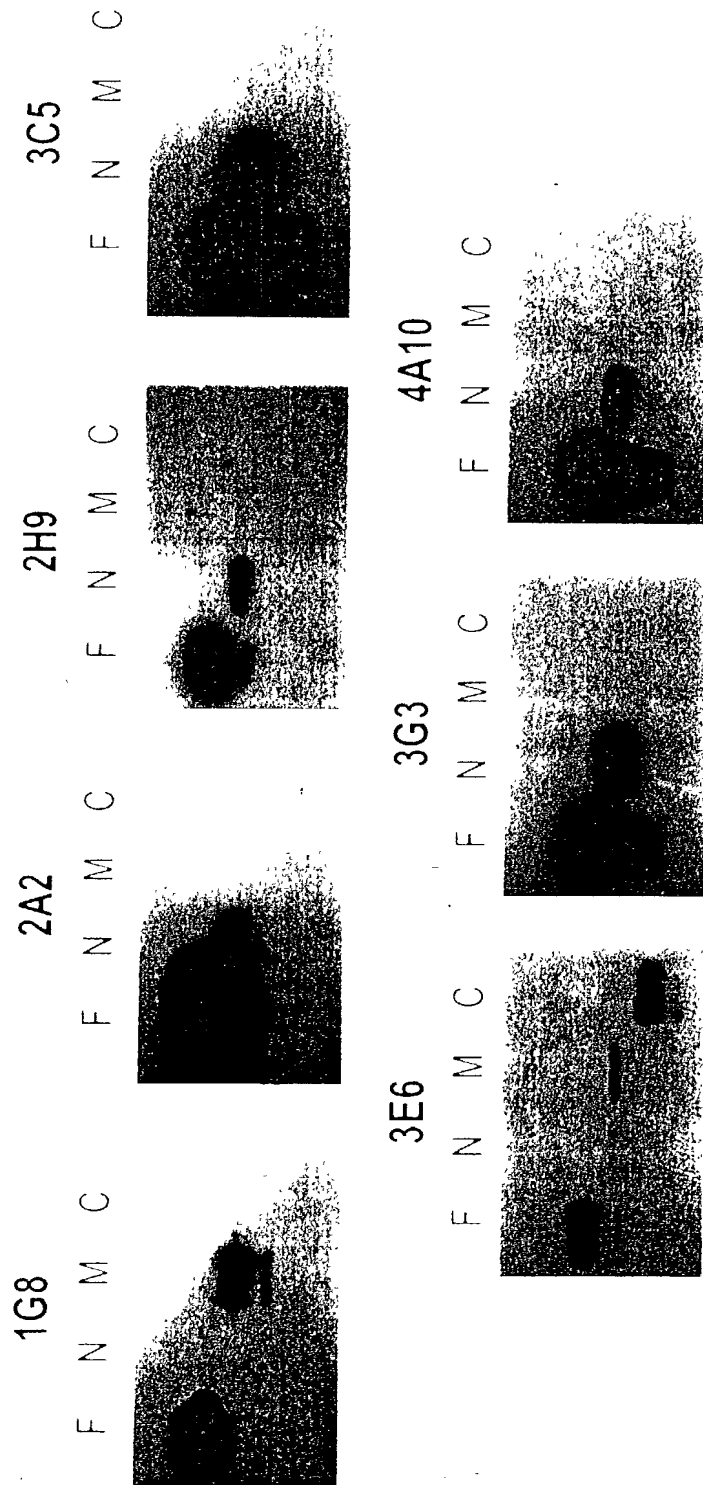
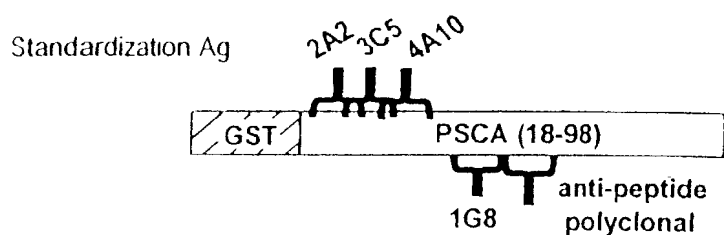
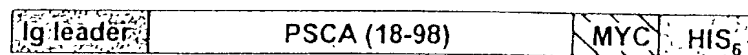
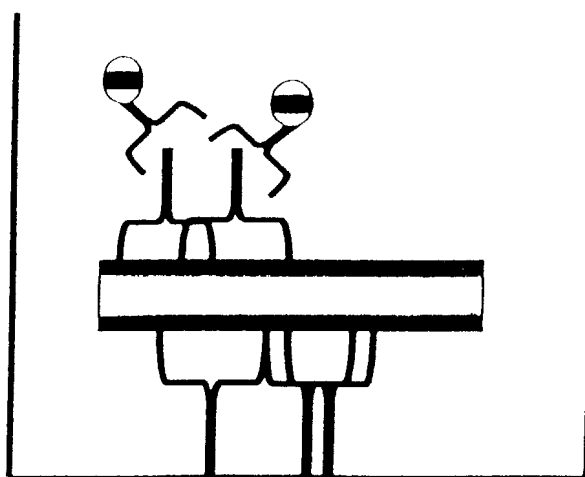


FIG. 50

A

Engineered mammalian secreted form

**B**

Anti-IgG2a HRP

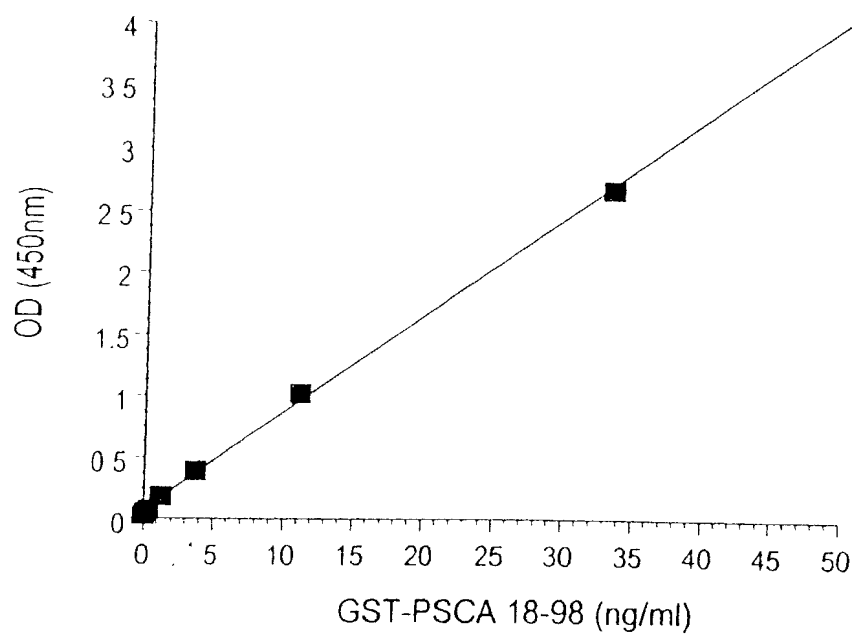
Anti-PSCA mAbs 3C5+4A10+2A2 (IgG2a)

PSCA

Affinity purified anti-peptide polyclonal
+ mAb 1G8 (IgG1)

FIG. 51

A



B

Sample	OD+range (n=2)	ng/ml
vector	0.005+0.001	ND
vector+hu serum	0.004+0.001	ND
secPSCA	2.695+0.031	32.92
secPSCA+hu serum	2.187+0.029	26.55

FIG. 52

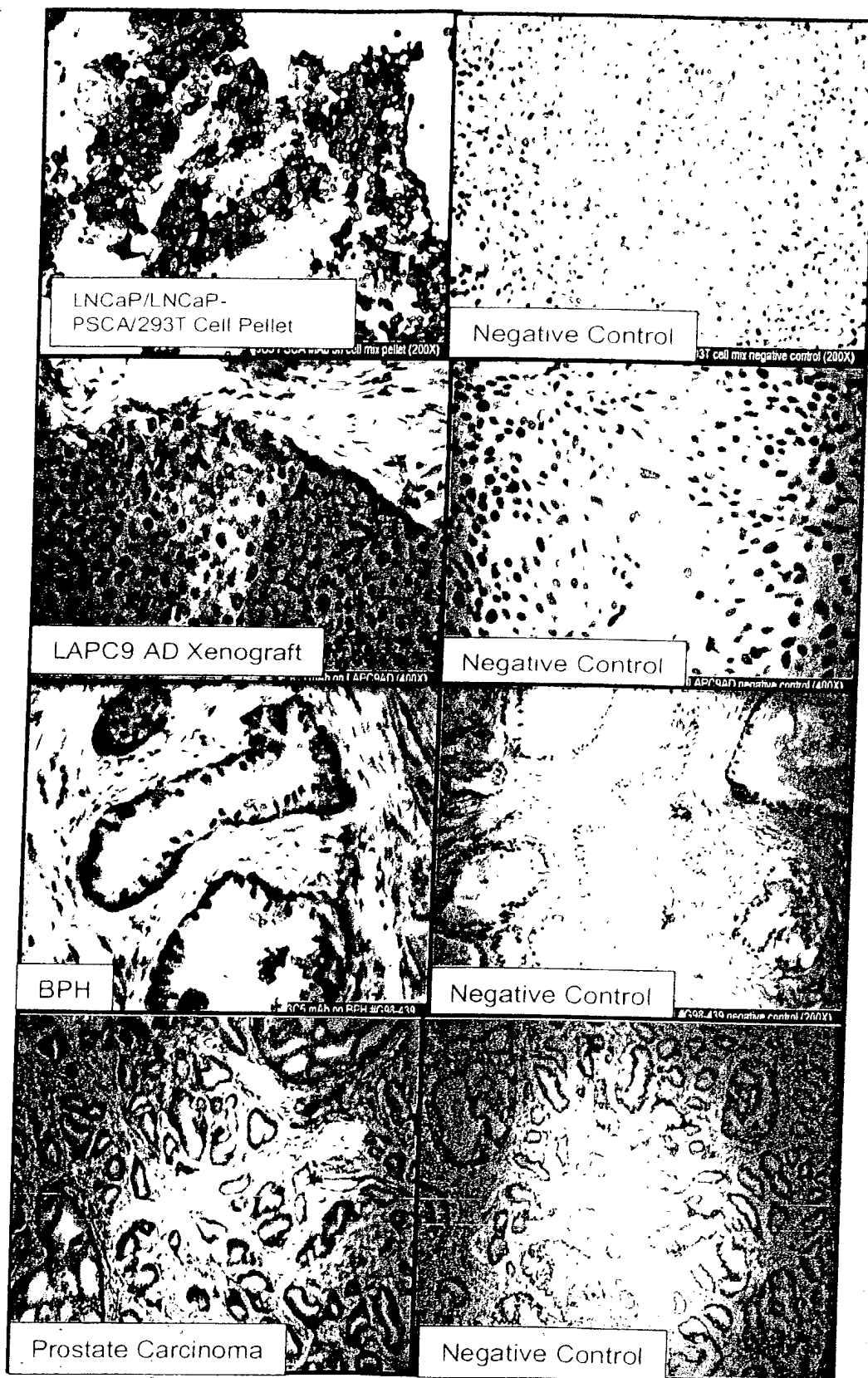


FIG. 53

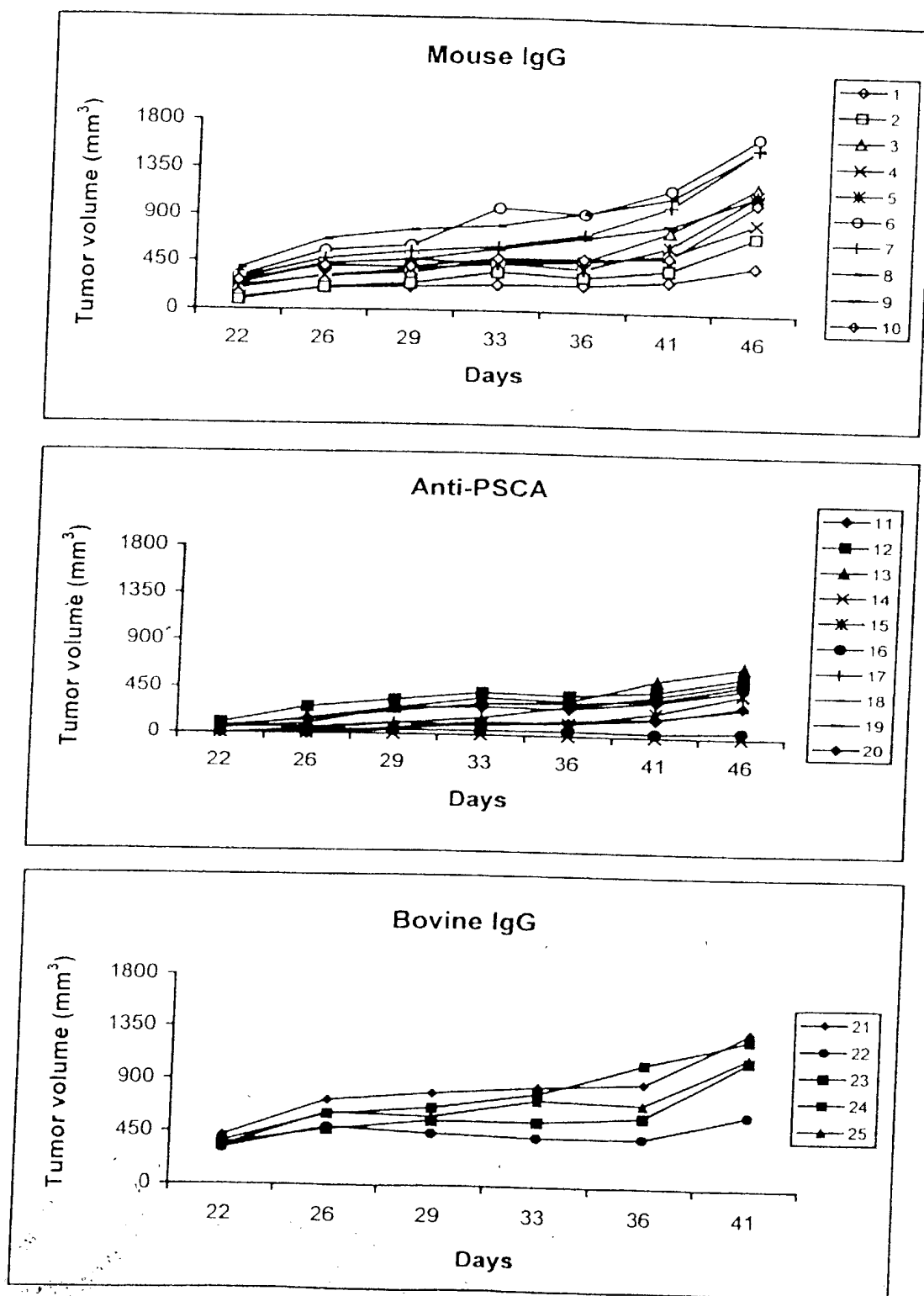


FIG. 54

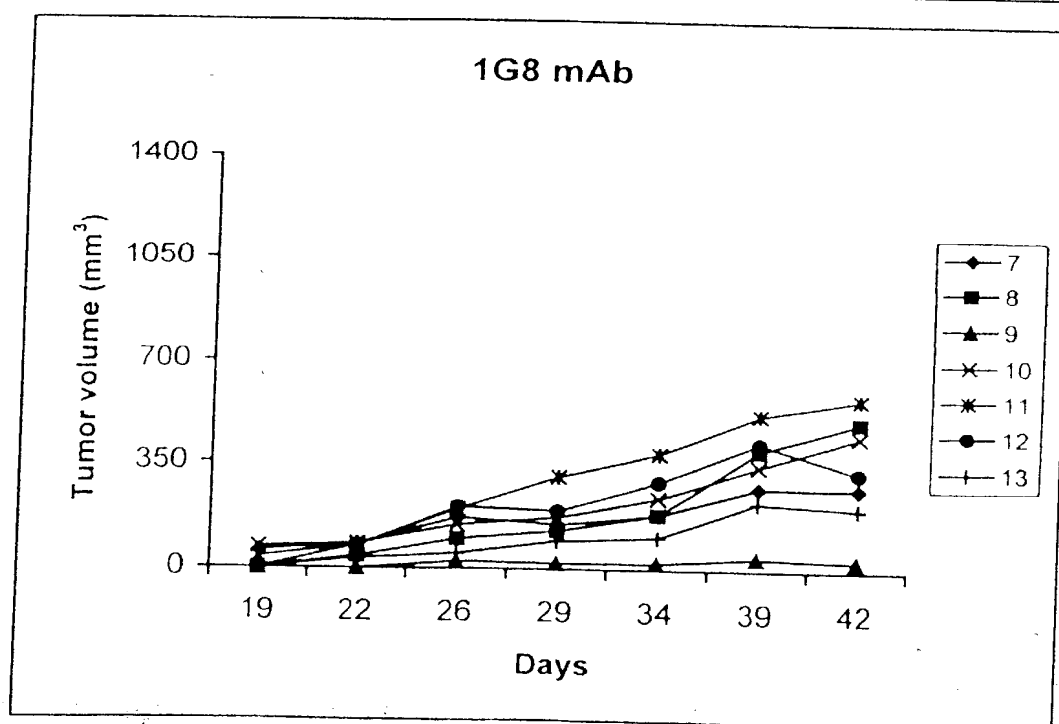
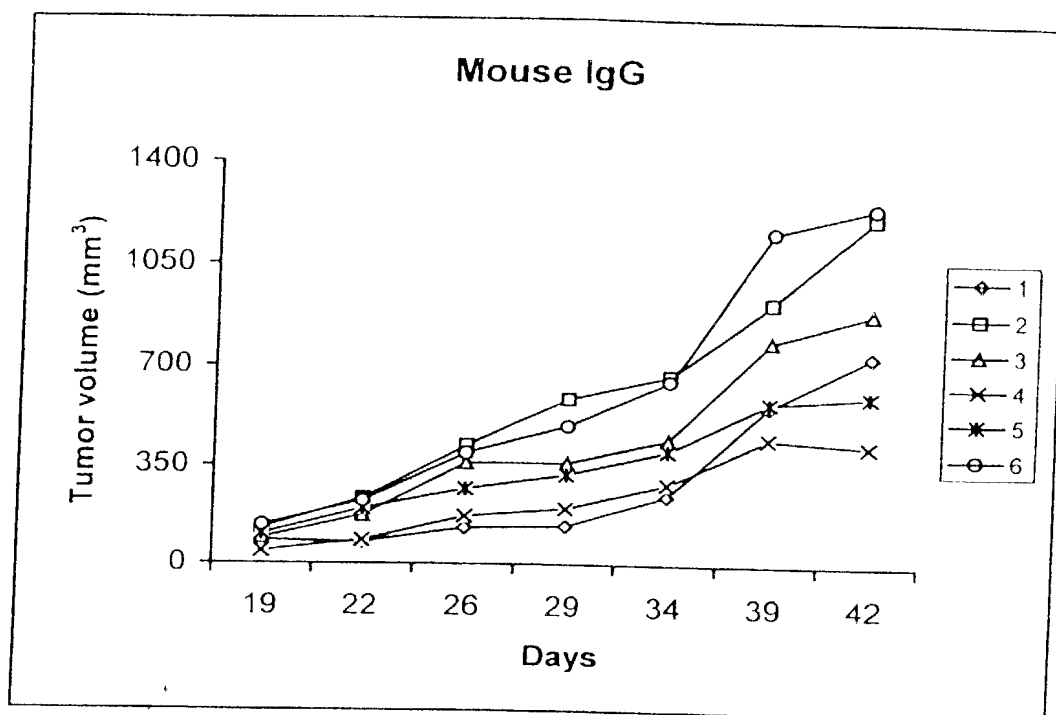


FIG. 55

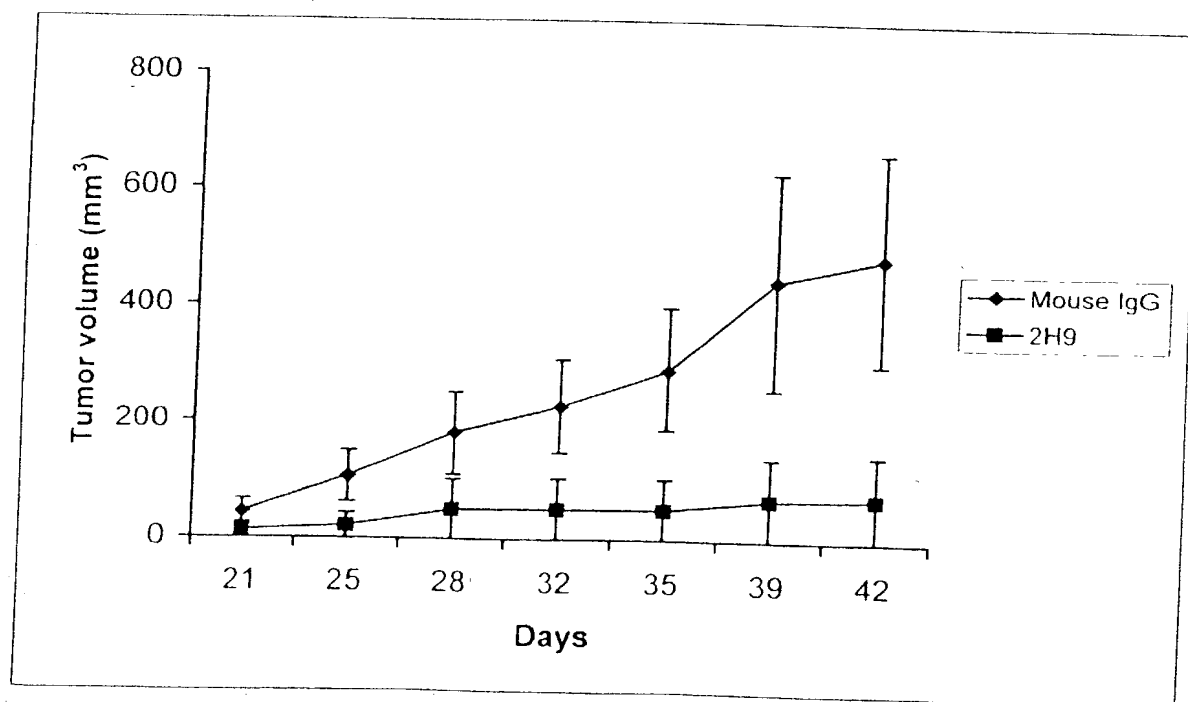
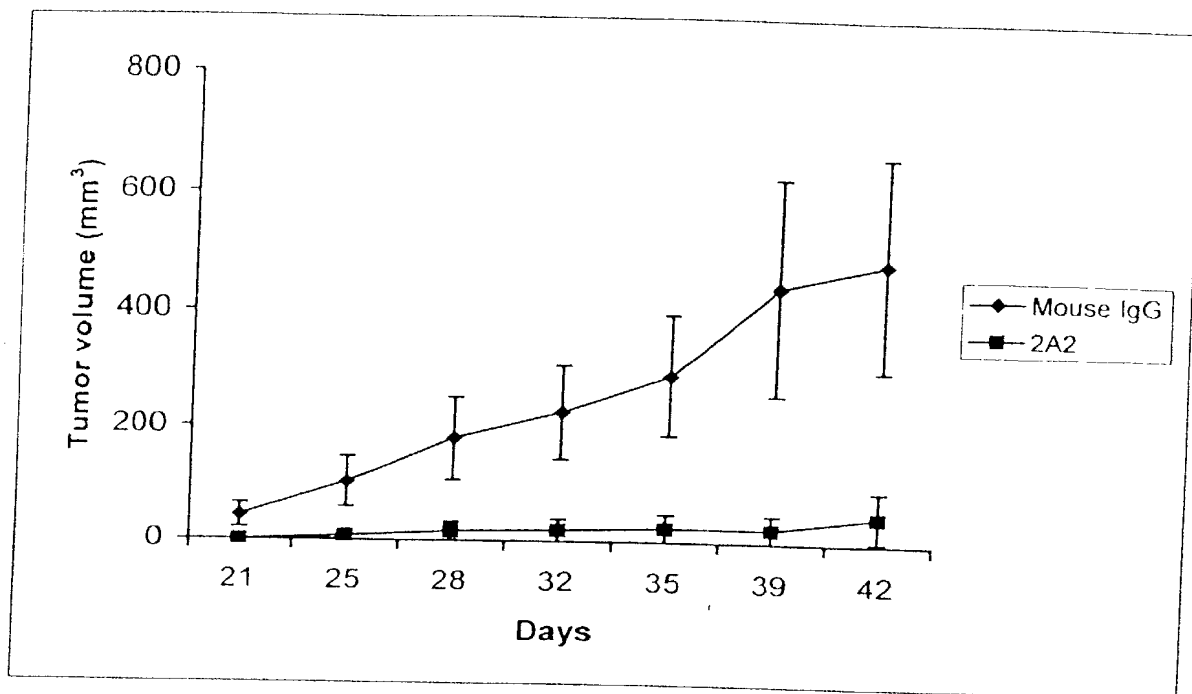


FIG. 56

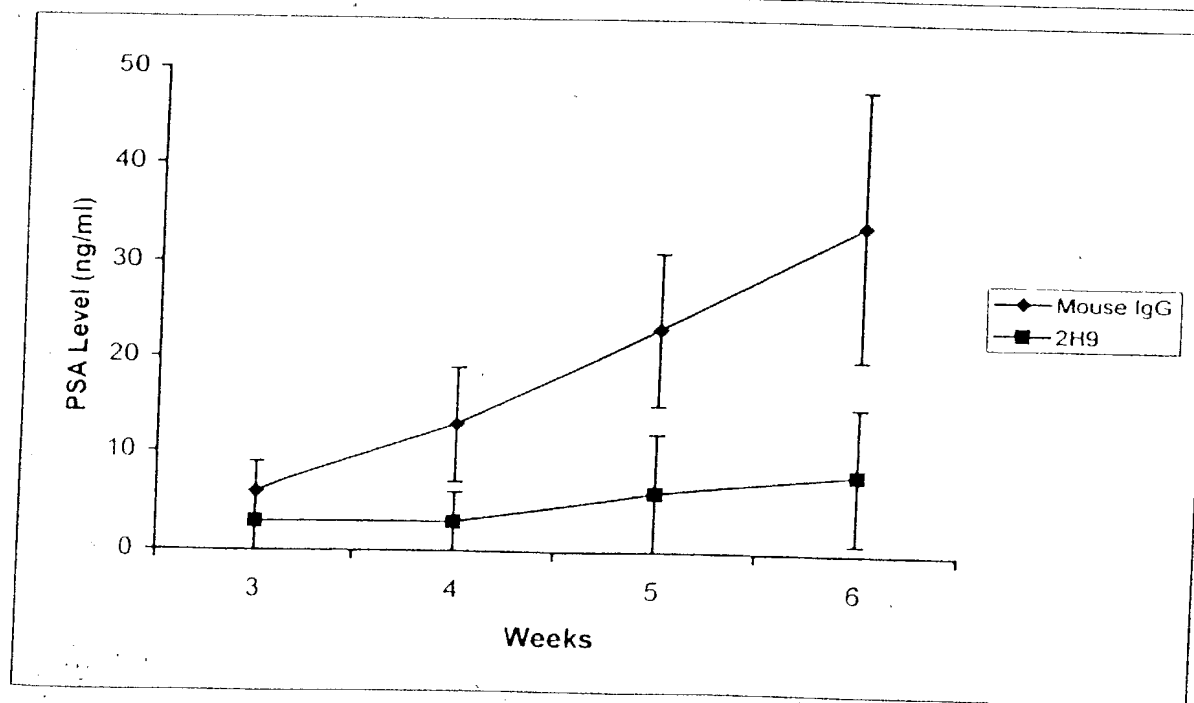
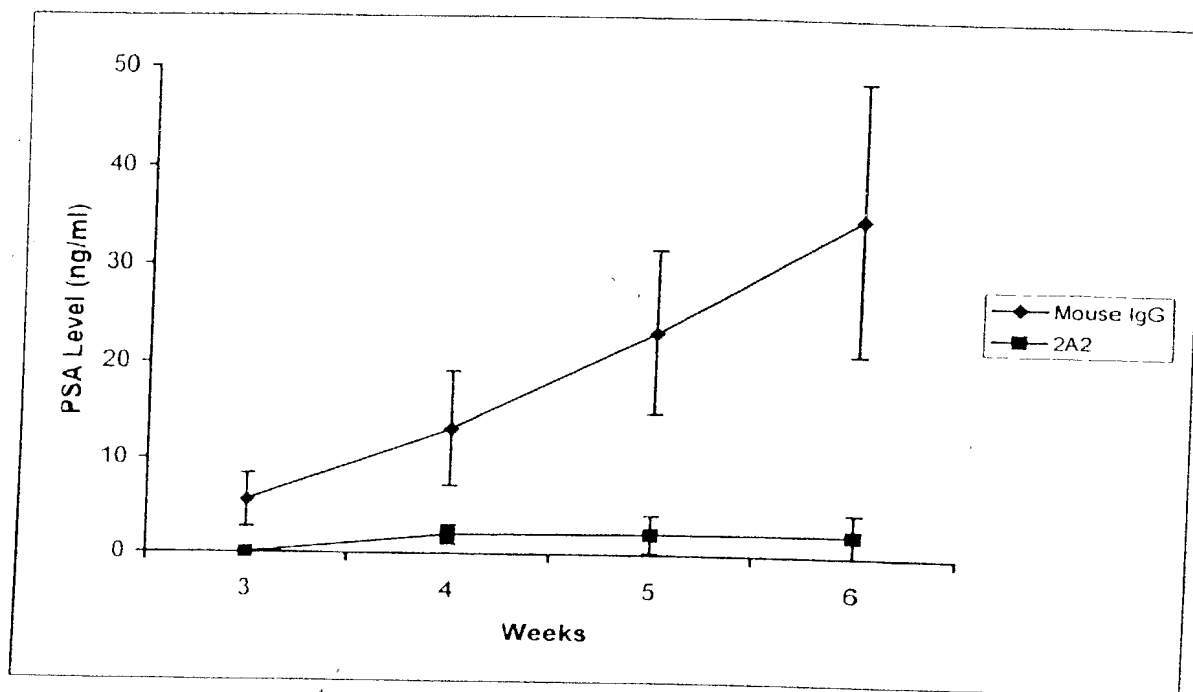
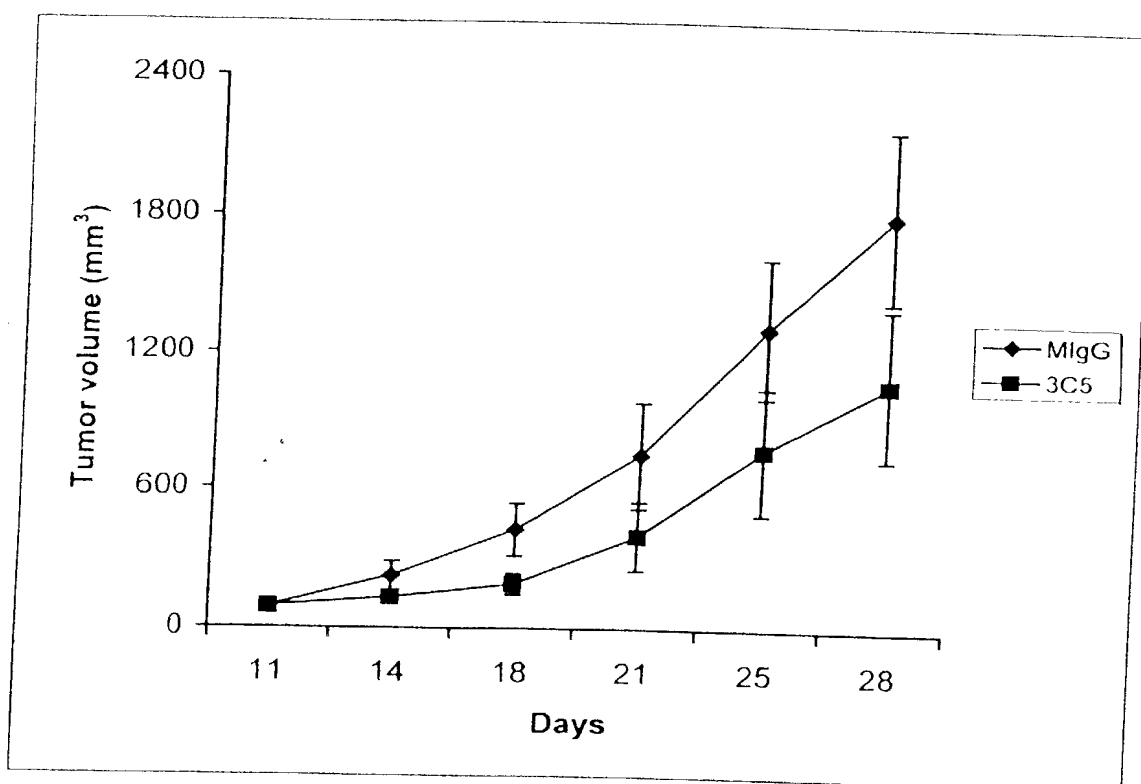


FIG. 57



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ETCACTGTCTCTGCAGCCAAACGACACCCCATCTGTCTATCCACTG
V T V S A A K T T P P S V Y P L

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CTGGCC
L A

FIG. 60

AATGACTTCGGGTTGAGCTGGGTTTTATTATTGTTCTTTTAAAAAGGGTCCGGAGTGAA 60
N D F G L S W V F I I V L L K G V R S E 20

GTGAGGCTTGAGGAGTCTGGAGGAGGCTGGGTGCAACCTGGAGGATCCATGAACTCTCC 120
V R L E E S G G G W V Q P G G S M K L S 40

TGTGTAGCCTCTGGATTTACTTTTCAGTAATTACTGGATGACTTGGTCCGCCAGTCTCCA 180
C V A S G F T F S N Y W M T W V R Q S P 60
CDR1

GAGAAGGGGCTTGAGTGGGTTGCTGAAATTCGATTGAGATCTGAAAATTATGCAACACAT 240
E K G L E W V A E I R L R S E N Y A T H 80
CDR2

TATGCGGAGTCTGTGAAAGGGAATTCACCATCTCAAGAGATGATTCAGAAAGTCGTCTC 300
Y A E S V K G K F T I S R D D S R S R L 100

TACCTGCAATGAACAACCTTAAGACCTGAAGACAGTGAATTTATTACTGTACAGATGGT 360
Y L Q M N N L R P E D S G I Y Y C T D G 120

CTGGGACGACCTAACTGGGGCCCAAGGACTCTGGTCACTGTCTCTGCAGCCAAACGACA 420
L G R P N W G Q G T L V T V S A A K T T 140
CDR3

CCCCCATCTGTCTATCCACTGGCCCCCTTGTA
P P S V Y P L A P C V

FIG. 61

CDR1 Comparisons

1G8	1gG _{1k}	Middle	G	F	N	I	K	D	Y	Y	I	H
2H9	1gG _{1k}	N-Term.	G	F	T	F	S	N	Y	W	M	T
4A10	1gG _{2ak}	N-Term.	G	Y	T	F	S	S	Y	W	M	H

CDR2 Comparisons

1G8	1gG _{1k}	W	I	D	P	E	N	G	D	T	E	F	V	P	K	F	Q	G		
2H9	1gG _{1k}	E	I	R	L	R	S	E	N	Y	A	T	H	Y	A	E	S	V	K	G
4A10	1gG _{2ak}	N	I	D	P	G	S	G	Y	T	N	Y	A	E	N	L	K	T		

CDR3 Comparisons

1G8	1gG _{1k}	G	G	F																
2H9	1gG _{1k}	L	G	R	P	N														
4A10	1gG _{2ak}	R	S	T	M	I	T	T	G	F	A	Y								

FIG. 62

A



B

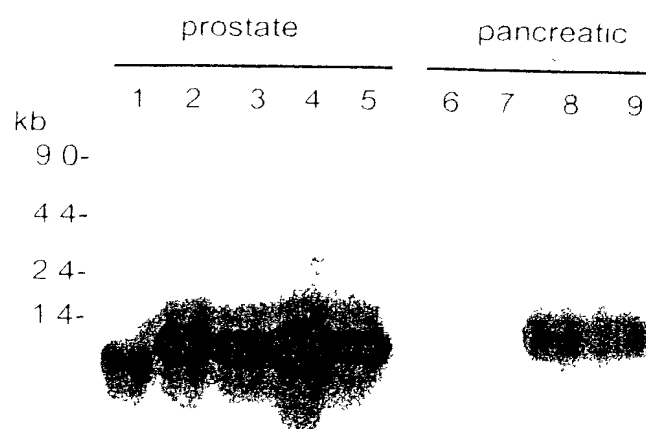


C



D

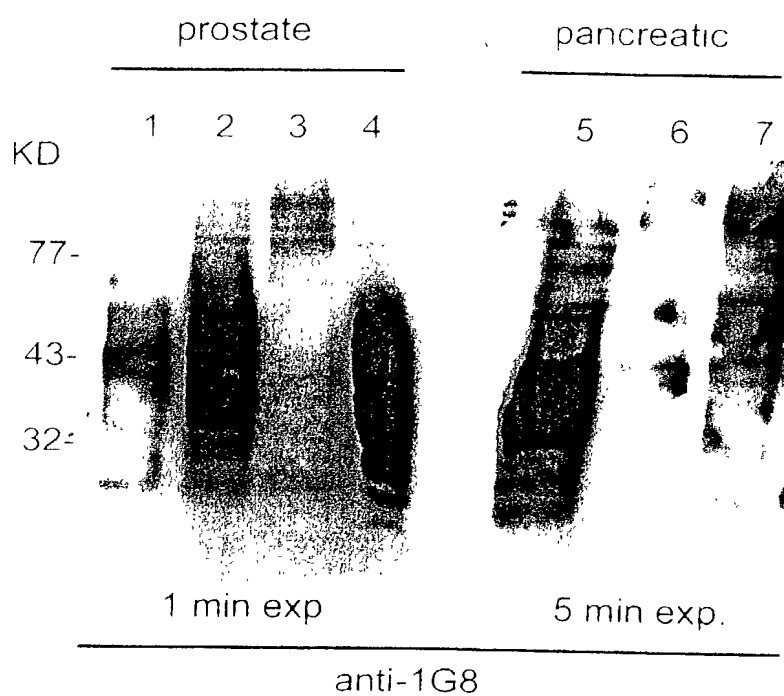




1. Prostate
2. LAPC-4 AD
3. LAPC-4 AI
4. LAPC-9 AD
5. LAPC-9 AI

6 PANC-1
7 BxPC-3
8 HPAC
9 Capan-1

FIG. 64



- 1 LAPC-4 AD
- 2 LAPC-9 AI
- 3 LNCaP
- 4 LNCaP-PSCA

- 5 HPAC
- 6 Capan-1
- 7 ASPC-1

FIG. 65

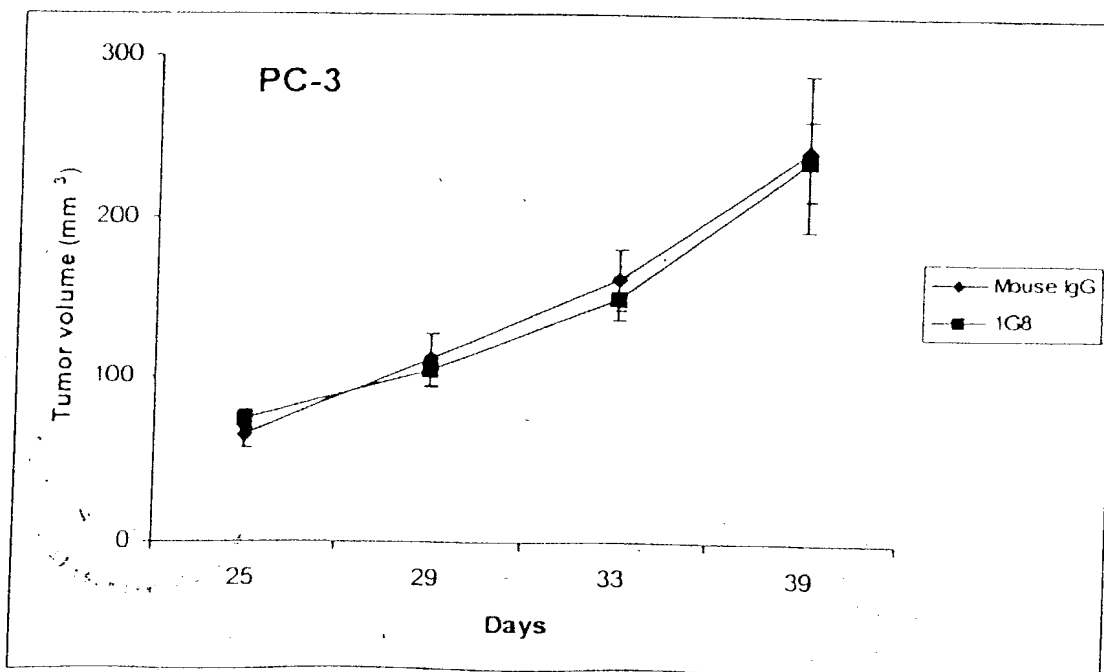
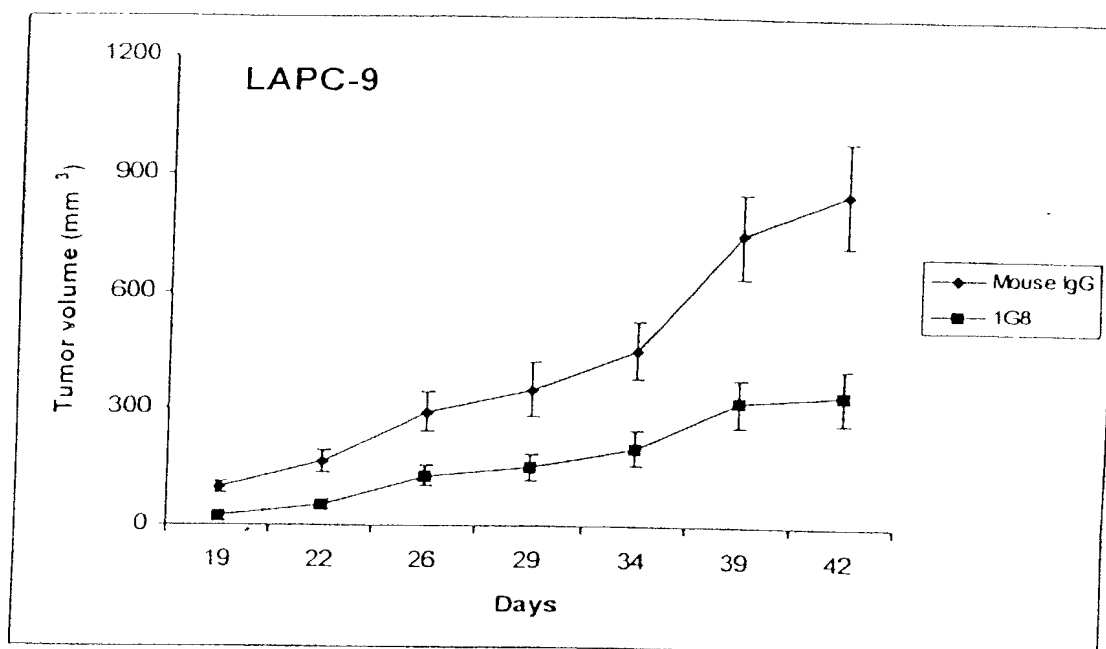
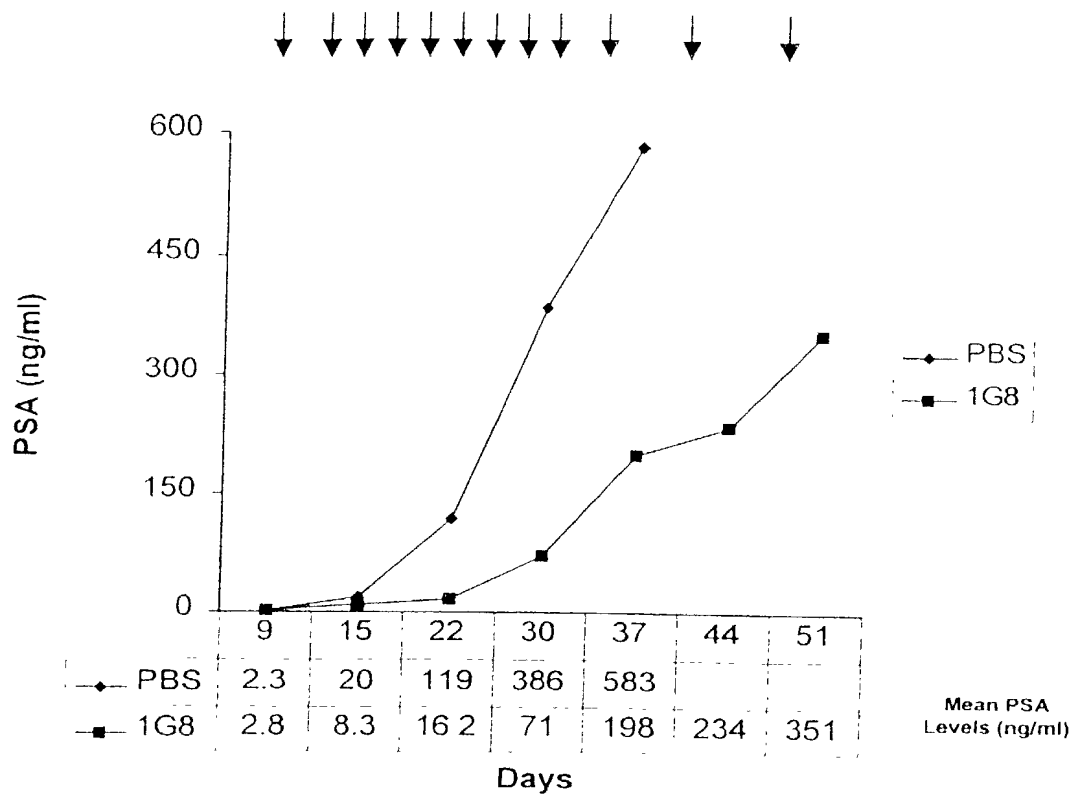


FIG. 66

A)



B)

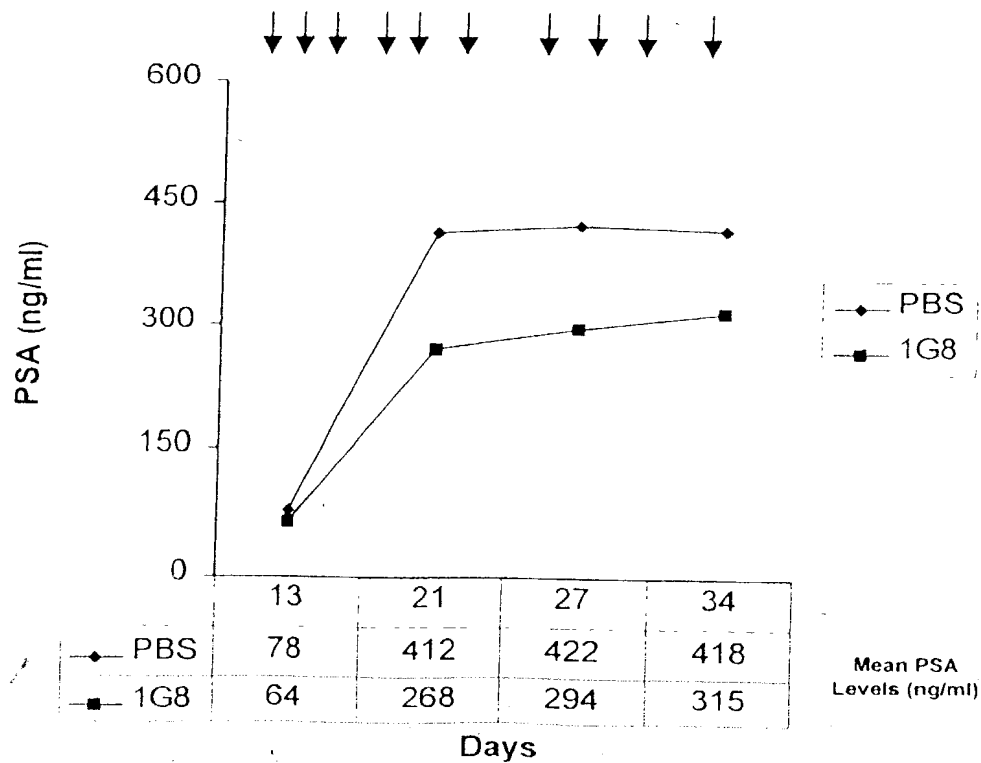
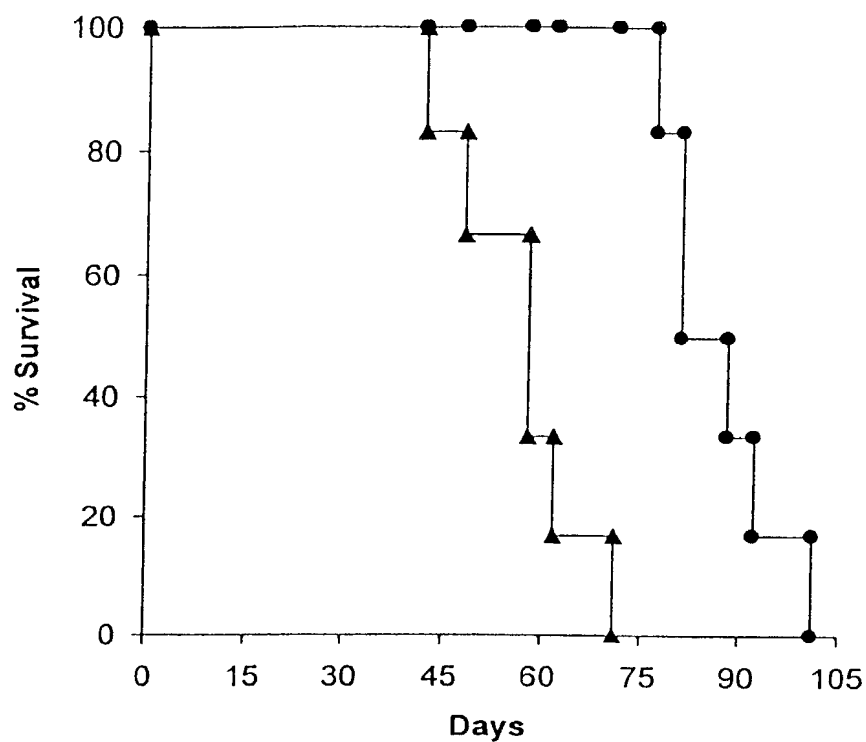


FIG. 67

A)



B)

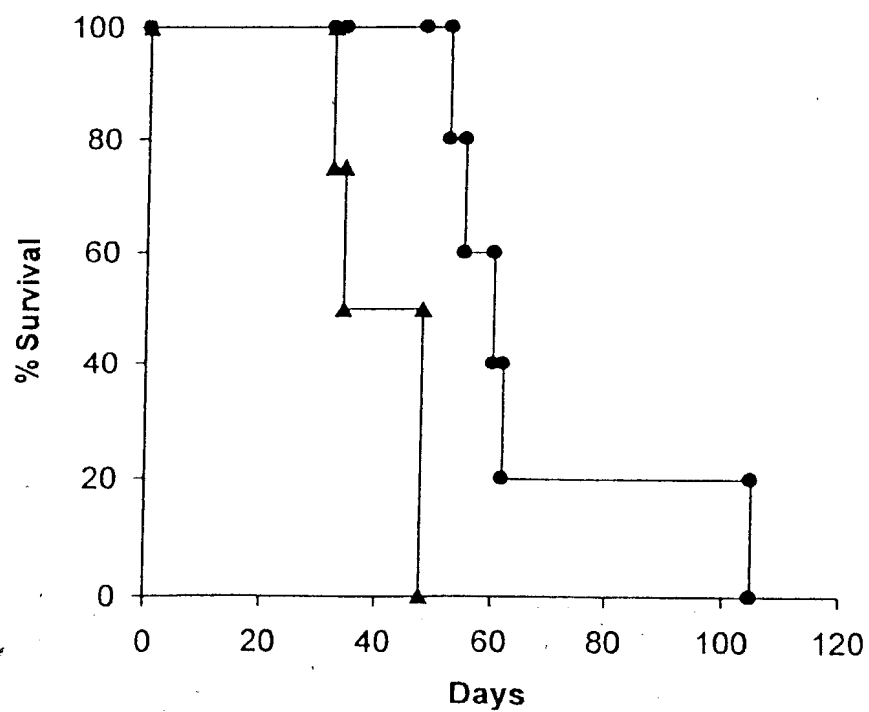
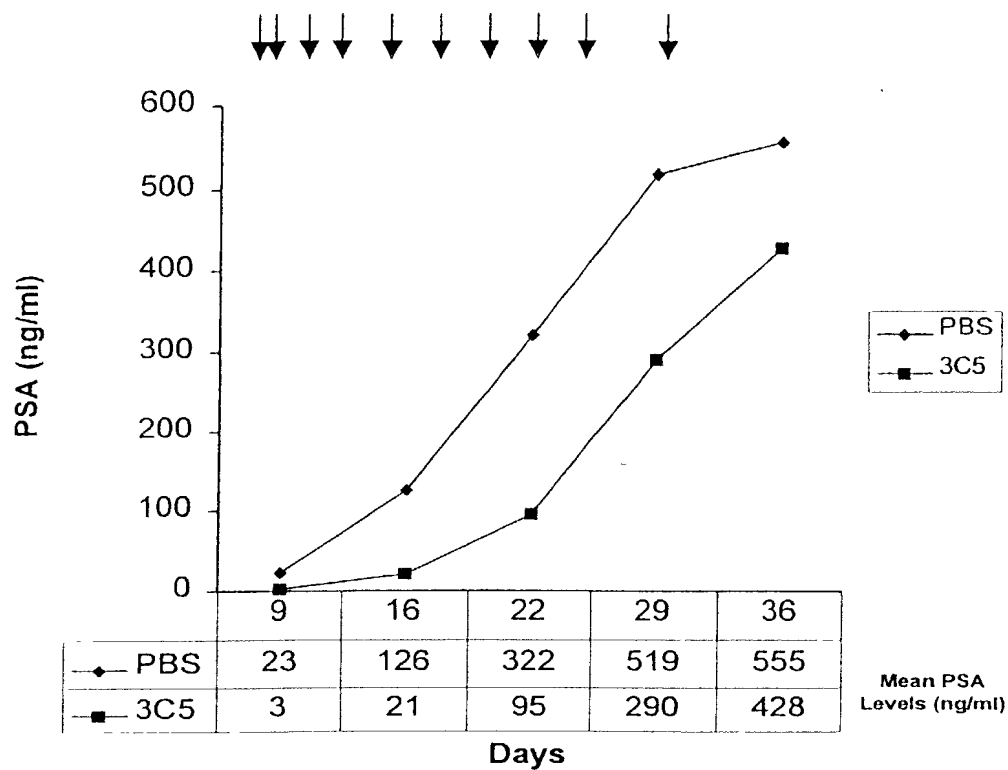


FIG. 68

A)



B)

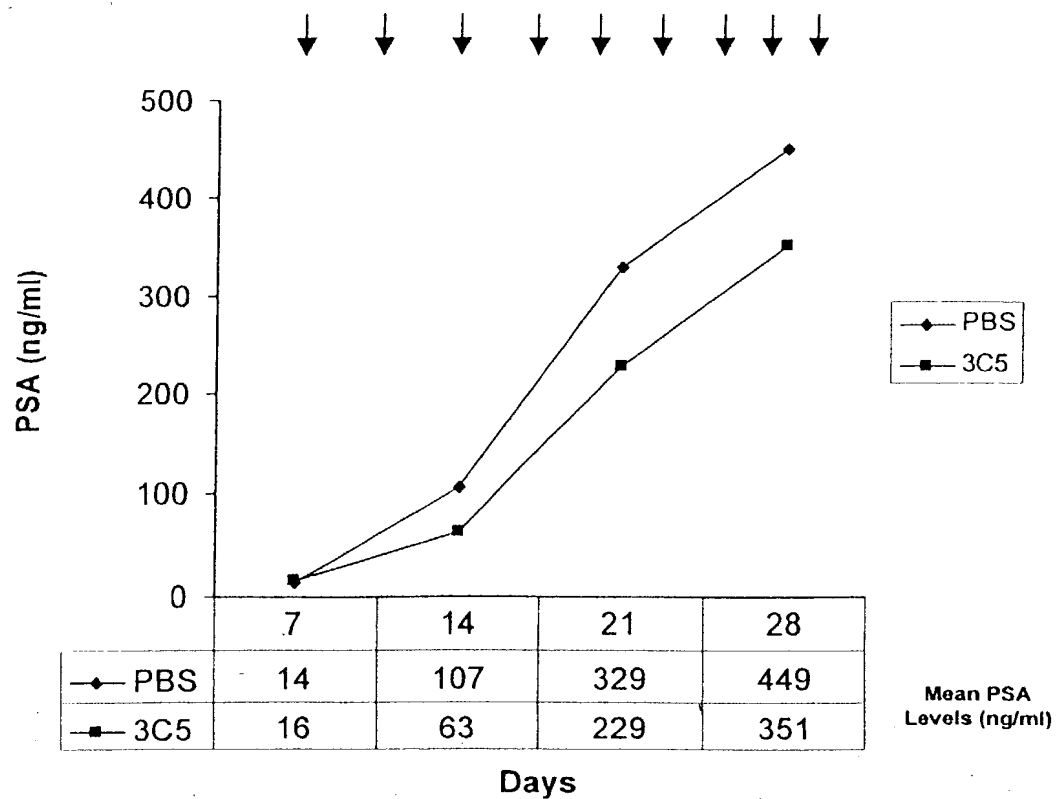


FIG. 69

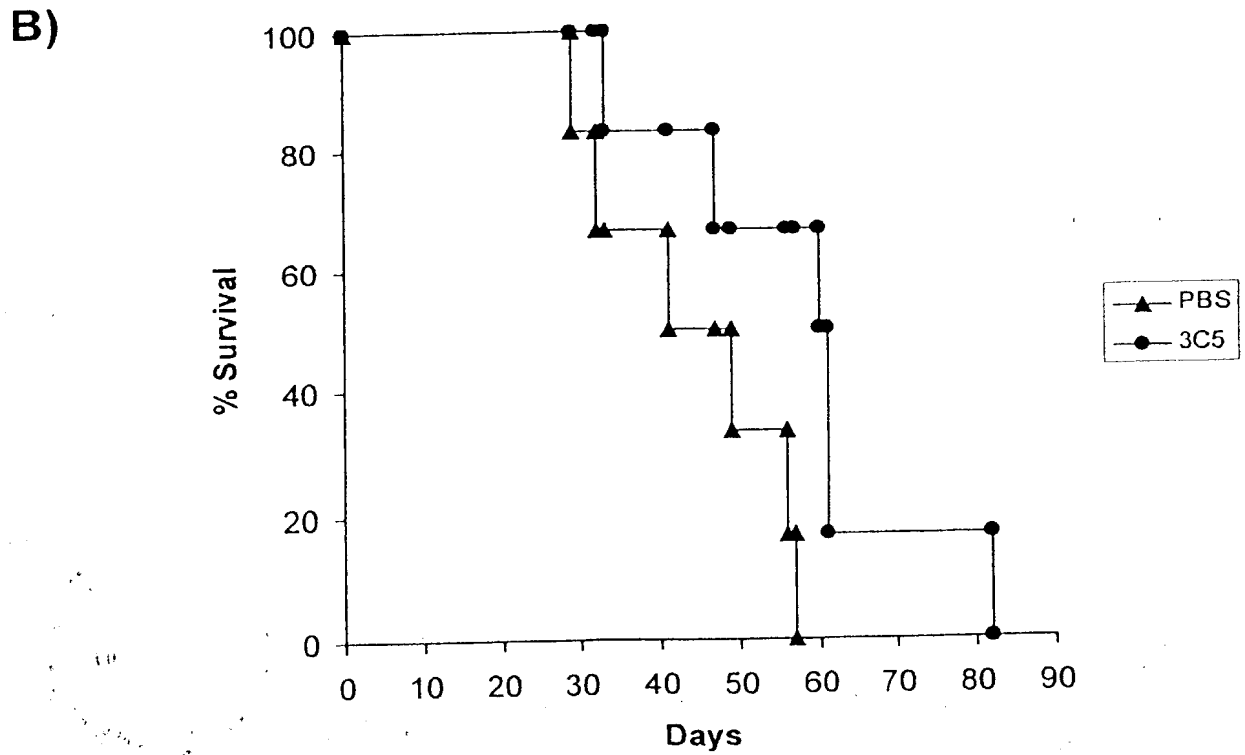
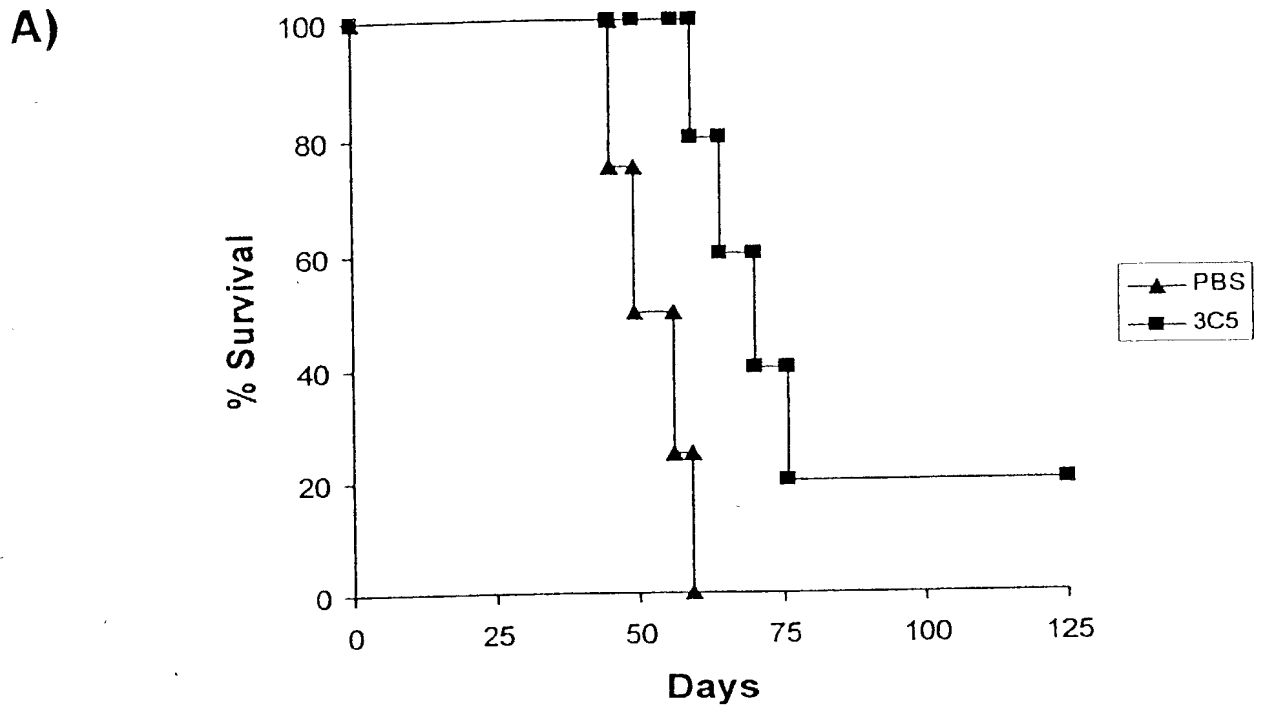


FIG. 70

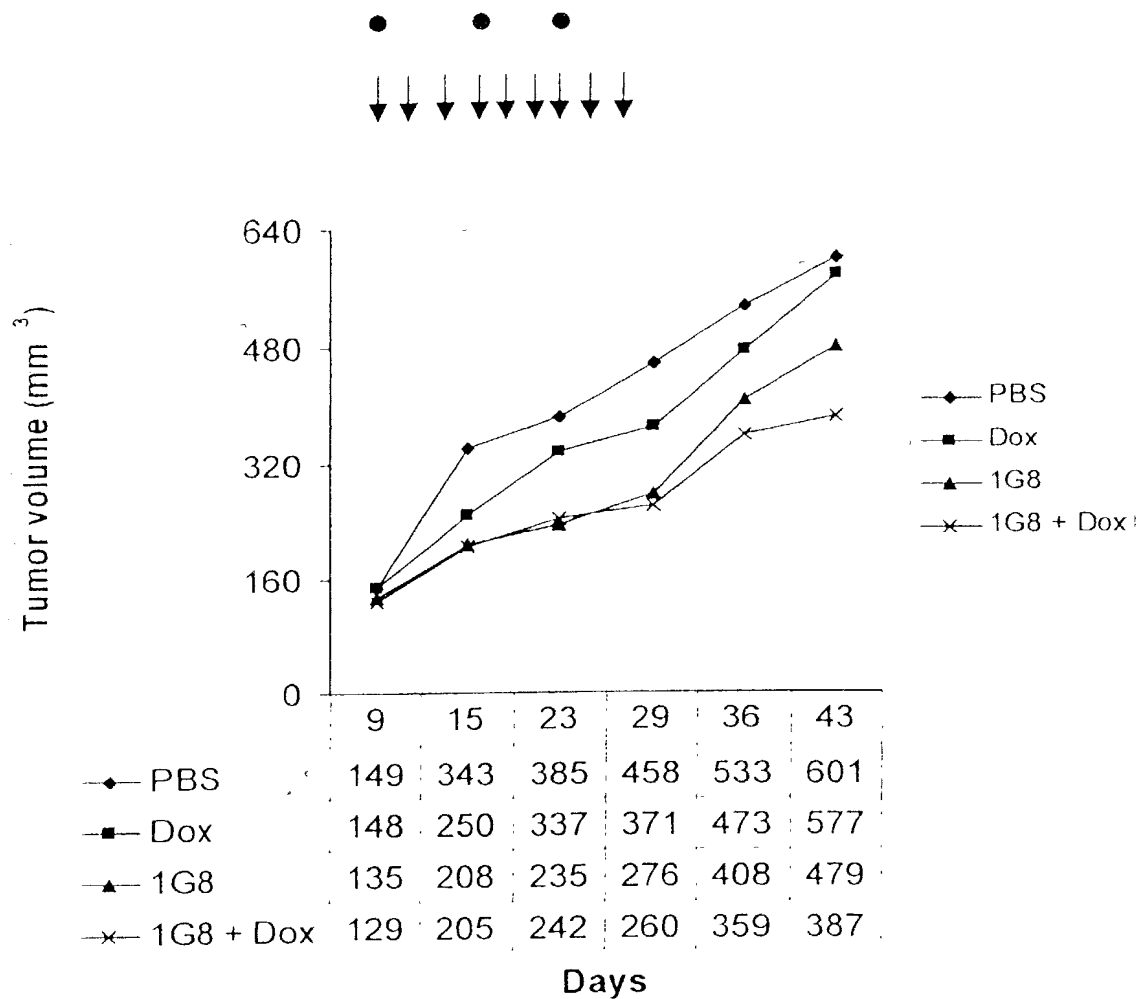
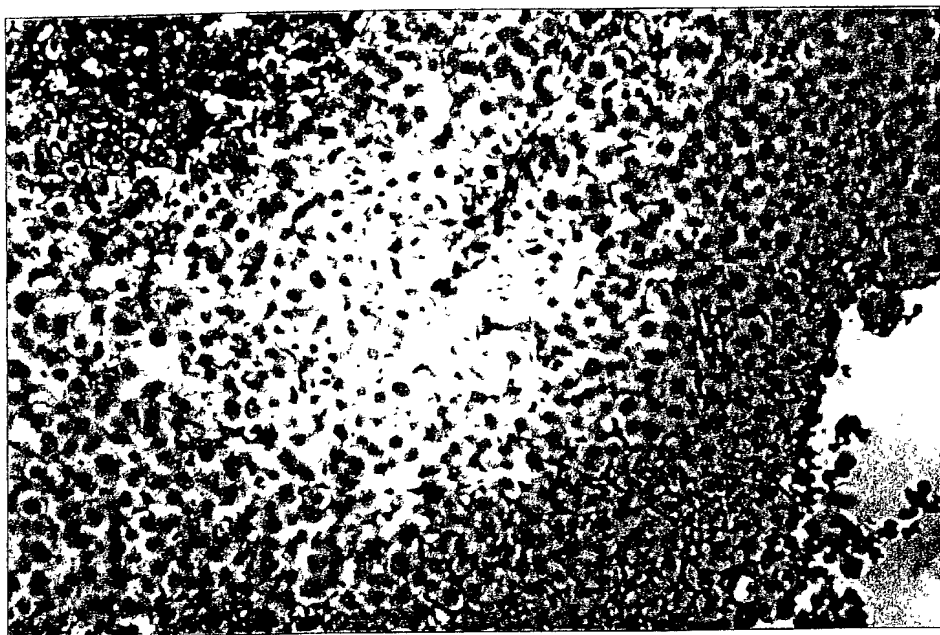


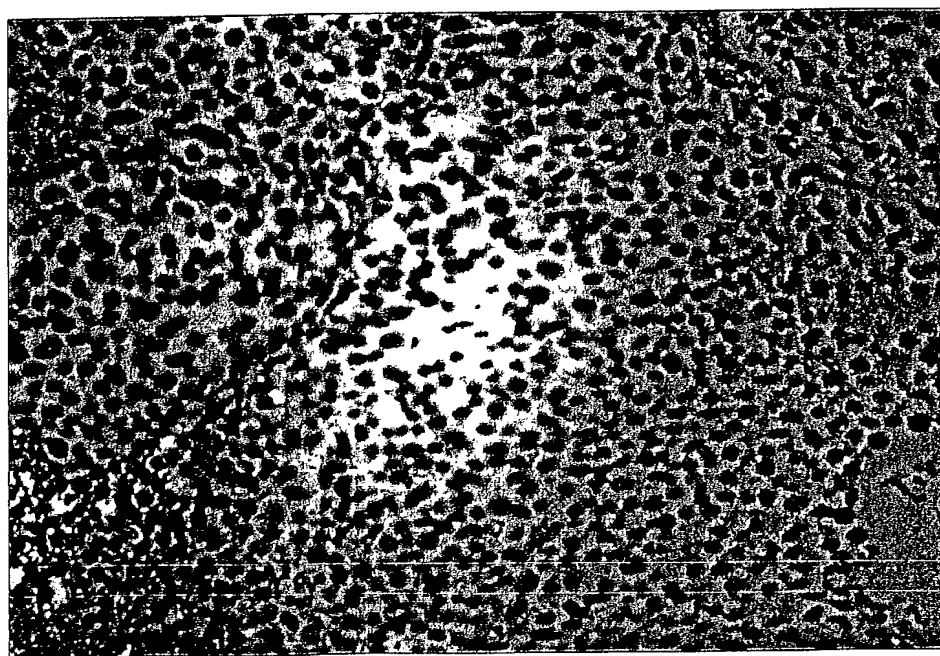
FIG. 71

PSCA 3C5 MAb Localizes within
LAPC9AD Xenograft Tissue

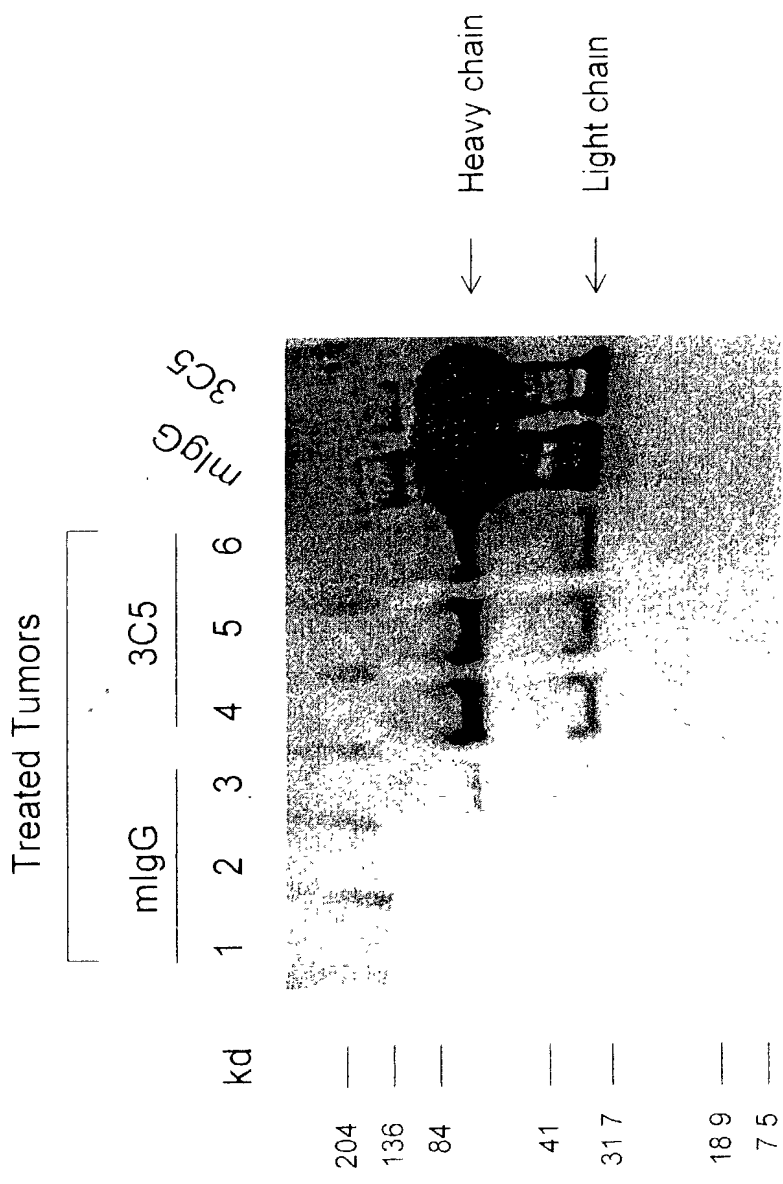
3C5 Treated



mIgG Treated



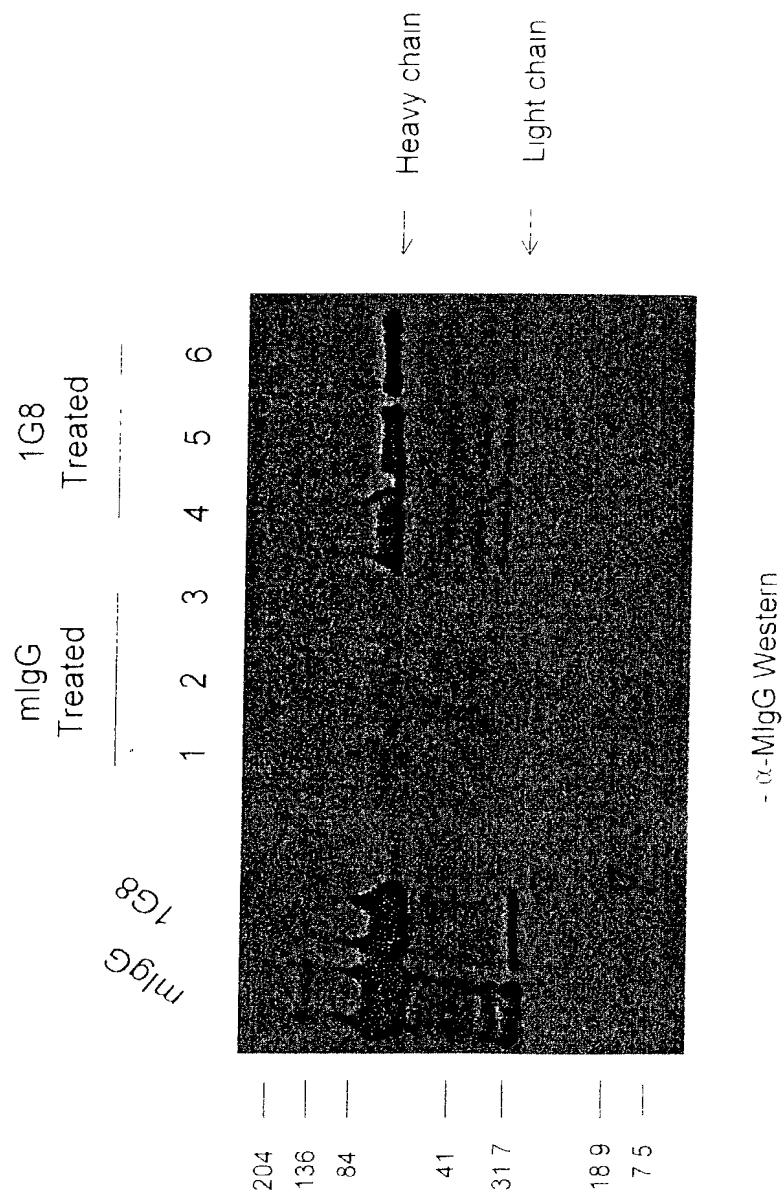
3C5 Anti-PSCA MAb is Localized to Established LAPC-9 Tumors



Western blot developed with α -mIgG/k

FIG. 72

SPECIFIC TARGETING OF THE 1G8 ANTI-PSCA MAb TO ESTABLISHED LAPC-9 TUMORS



Method: Mice bearing established LAPC-9 tumors ($>100 \text{ mm}^3$) were injected with either mIgG or the anti-PSCA MAb 1G8. Tumors were harvested a week later and made into protein lysates for Western analysis.

FIG. 73